nature

3 December 1981

Tolerance but no quarter for creationism

Creation-science goes on trial next week in Arkansas. The issue is whether recent legislation establishes religion. It would be better that it should have been the freedom of teachers to say what they believe.

The trial which opens in Arkansas next Monday on the state's decision to legislate for equal time in the school curriculum for creationism and Darwinian evolution will not be as dramatic an occasion as the trial of the Tennessee biology teacher, Mr John Scopes, in 1928. Then the issue was whether a teacher could be prevented by the law from telling his students what Darwin said. (Technically, Scopes was found guilty and fined $100, but his lawyer Clarence Darrow made such a monkey of the prosecution that the conviction was quashed by the United States Supreme Court.) Now the argument is about a much narrower constitutional issue. By requiring equal time for what is called "creation-science" with Darwinism, is the state of Arkansas violating the provision of the United States constitution that ensures the separation of religion and the state? The American Council for Civil Liberties, which is to its credit bringing suit against Arkansas, has chosen to argue that what is called creation-science, itself the creation of predominantly religious people, cannot be made a compulsory part of the curriculum in state schools without institutionalizing religion.

The issue is far from clear cut. Would it necessarily be a constitutional offence if a state board of education required schools to teach the early history of the Christian Church, plainly a matter of great cultural importance? Part of the weakness of the state's case is that under the "Balanced treatment for creation-science and evolution-science Act", rushed through the state legislature in March this year, the doctrine of equal time is vested in the law, and is not an administrative decision of the board of education. But the essential flaw, likely to fall outside the terms of reference of next week's trial, is the sheer folly implied by the term "creation-science".

Three separate questions arise — the status of Darwinism, the status of "creation-science" and the question of how the components of any school curriculum should be decided. The argument next week will be dominated by attempts to demonstrate that Darwinism and "creation-science" are equally valid explanations of the origin of the natural world. The state attorney-general's decision to call as a witness Professor Chandra Wickramasinghe, Sir Fred Hoyle's collaborator in the theory of life from outer space, is plainly meant to show that even among non-creationist scientists, Darwinism does not have the field to itself. The misfortune in the framework of next week's trial is that there will be no opportunity to challenge the assumption, not only in Arkansas, that state governments can sensibly intervene on specific matters of educational policy.

The status as a scientific theory of Darwinism was predictably well defined by Sir Andrew Huxley in his anniversary address to the Royal Society this week (see page 395). The supposed conflict on which the creationists have seized between the supposed gradualism of Darwinism and the observation that evolution may be episodic (chiefly due to S.J. Gould and N. Eldridge) is not a conflict at all. Gould will be saying just this at next week's trial. Neither is the problem of the "missing links" a problem. Darwinism has all the attributes of a mature and powerful scientific theory — newly discovered and apparently contradictory phenomena such as Mendelian inheritance have turned out to be confirmatory. And with the passage of time, the once perplexing question of how living things came to be the way they are has been particularized to a group of more tangible (if as yet unanswered) questions, the regulation of embryogenesis and the evolutionary role of consciousness for example.

During the long correspondence on the role of the British Museum (Natural History) in the past year, much has been made of Sir Karl Popper's confusing remark that the theory of evolution is not falsifiable and must therefore be called "metaphysical". Like cladism in its own field, that is a useful way of classifying scientific theories, suggesting what kinds of tests may be considered compelling. But none of this is relevant to the function of the theory of evolution in scientific enquiry. What matters there is that the theory of evolution is found empirically — by biochemists as well as simple biologists — by planning investigations and designing experiments. To pretend that the theory carries no compelling conviction while problems remain unsolved is as ridiculous as it would be to hold that Newtonian mechanics (within its accepted field of validity) will remain unproven so long as the general three-body problem has not been solved.

Creationism, even when dignified as "creation-science", has a quite different status. Many working scientists, finding that it does not help in ordering thought or designing experiments, are tempted to dismiss it as rubbish. Individually, they are right to do so. Collectively, however, the scientific profession would be mistaken to seek to outlaw creationism. Many religious people find creationism a useful way of ordering their own non-scientific thoughts. Some religious Darwinists are also creationists, suggesting that the alternative ways of accounting for the natural world are orthogonal ways of ordering phenomena. But the scientific community's justifiable complaint against the resurgence of creationism in the past few years is that it is now called creation-science. For what will be given equal time in the Arkansas curriculum is not science at all in the sense of being a system for relating all phenomena in a certain domain to a single set of causes which are themselves susceptible to investigation. Toleration of this shoddy pretence has already gone too far, and in surprising places. (*Nature*'s complaint, earlier this year, about evidence of creationist backsliding at the British Museum, is not sufficiently answered by Sir Andrew Huxley's assertion, earlier this week, that the signs were "so slight that most visitors do not even notice them".). The plain truth, which museum exhibitors and state legislators must recognize, is that creationism is not a part of science but an alternative to it.

However the trial next week is decided, the question will remain unresolved of how the curriculum in state schools should be decided. Improbably, the creationists may lose their case, but the right of state governments to specify the content of the school curriculum will be unchallenged. On paper, that right has a simple derivation. State schools are dependants of the state government, and those who teach in them are employees of their local governments. On the principle that public employees must be accountable to their paymasters for all aspects of their work, it is possible to argue that a state is well within its rights to require that teachers should teach whatever is required of them. So would a tobacco-growing state such as Virginia or North Carolina be allowed to instruct its teachers to play down the evidence for a link between smoking and lung cancer? States' rights apart, such a decision, however constitutional, would provoke an outcry that could not be gainsaid. And the American Council for Civil

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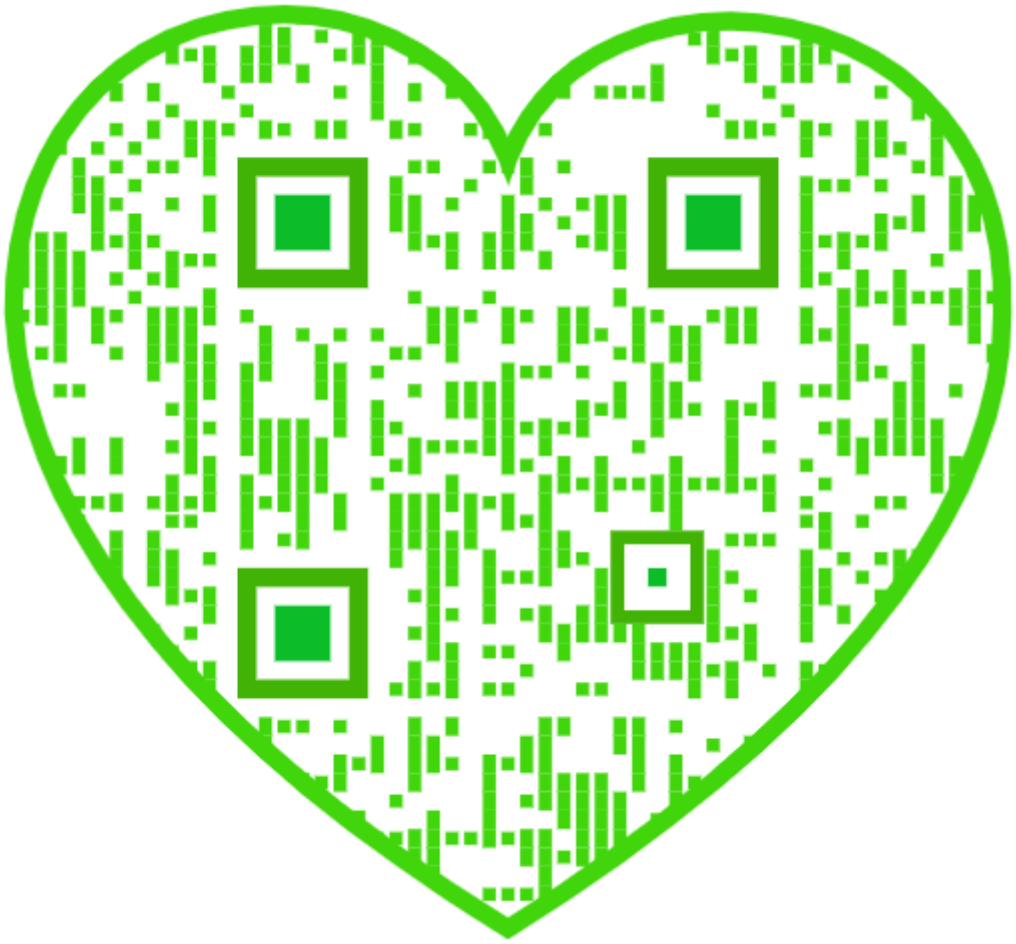

Liberties would then be filing suit on the grounds of the First Amendment to the Constitution, that which assures free speech — in this case the teachers'.

Exactly the same argument applies to the disastrous legislation that the state of Arkansas has hung around its neck. (In the past few weeks, Mississippi has followed suit.) By requiring that biology teachers should in future teach "creation-science", it is requiring that honest people should tell what they consider to be lies in public. By doing so, Arkansas will undermine the professional integrity of a substantial part of the state's teaching staff and ultimately of the education system as a whole. Is it too much to hope that, when the trial is over — and however it is decided — Arkansas will have the wit to find some way of delegating the fine control of the school curriculum to those on whom the responsibility properly falls — the educationists employed for that purpose?

What systems analysis?

Laxenburg has a new director but an uncertain future. It also needs a policy.

Not so long ago, in the early 1970s, governments throughout the world were being asked to subscribe to what was intended as a unique instrument of scientific collaboration between East and West — the creation of the International Institute of Applied Systems Analysis at Laxenburg near Vienna. How splendid, the prospectus read, that there should be a research institute (supported principally by the governments of the Soviet Union and the United States) dedicated to the application of objective methods of analysis to problems of contemporary importance throughout the world. Eventually, the misgivings of those who held that the prospectus put the cart before the horse — the objective of East–West collaboration before the definition of a tangible programme of research — were overcome. The institute came into being and has indeed been a place where people, mostly modellers, desk scientists and even social scientists from both sides of the European boundary, have worked alongside each other. The most conspicuous product of this effort has been the study on energy published earlier this year. Now, for two reasons, the institute is in for a sea-change.

The most immediate difficulty is financial. President Reagan's first budget in March deliberately required that the National Science Foundation should reduce spending on overseas activities in general. Nobody appears at the time to have appreciated that United States government support for the Vienna institute is laundered through the National Academy of Sciences until the academy pointed out that it would be unable to pay the subscription due this month. The United States Administration has now found the funds with which the academy can make the contribution legally required of it. Whether it will be able to remain a member in 1983 remains uncertain — and will not be known until the budget for 1983 is published next February. It is, however, a fair guess that if the United States if forced to withdraw, the Soviet Union (whose financial contribution is identical) will also do so. The Laxenburg institute, already somewhat shrunken, must live with uncertainty until the summer.

At first sight, this may seem the worst time for a new director to take charge at Laxenburg. That, however, is the opposite of the truth. If change of some kind is unavoidable, a new director may be an advantage. This is the spirit in which to regard the appointment of Professor C.S. Holling, an ecologist from the University of British Columbia, to Laxenburg with effect from 1 December. he may have to share some of the personal anxieties about the future that afflict the staff of the institute, but he is likely to be less strictly bound by past promises than his predecessors would have been. With a little luck, he may be able to devise a programme of research that can be tailored as the months go by to suit whatever budget becomes available in the year ahead.

But that kind of programme should that be? The trouble so far at Laxenburg is that the original scepticism about the institute has not been stilled by its achievements. The institute has laboured for a decade and produced not so much a mouse as an elephant — a turgid account of familiar problems in the supply of and demand for energy that might have been illuminating if it could have been published several years earlier. Plainly that is a model for Dr Holling to avoid.

The question remains, however, of what is to be understood by the term "systems analysis" in the institute's title. Computer engineers have a simple answer, but the Laxenburg institute is intended as more than a computing centre. Originally, the phrase may have provided a seemingly innocuous unbrella beneath which people from different economic systems could work together. Now it may be necessary to acknowledge what has always been the truth — that the most useful problems for the institute to tackle are problems that impinge on economics and thus, because economics is the theory of social choice, on politics. What mechanisms should there be, for example, for sharing technological activity among industrialized states, the contemporary equivalent of Adam Smith's classical problem? How most efficiently can the supplies of natural gas to the industrialized world be used? And what can be said about the pattern of manufacturing industry and of world trade in the years ahead? With its unique constitution, the institute is well placed to capture international attention with attempts to answer such unspoken questions. The danger is that it will settle for yet another easy option, and devote itself to important but distant questions such as the problem of the accumulation of carbon dioxide in the atmosphere.

Yellow rain too soon

The United Nations commission on poisonous yellow rain in South-East Asia issued an inconclusive report. It would have been wiser not to comment.

The United Nations has done itself a power of harm by its appointment of a commission to investigate charges by the United States that the Soviet Union has been using biological weapons in South-East Asia and in Afghanistan. Last week, the commission (having been to South-East Asia but not to Afghanistan) said that it had been unable to reach a definite conclusion. This week, the commission (which would no doubt prefer to be released from its responsibilities) is likely to be asked by a formal resolution of the General Assembly to soldier on. Dr Kurt Waldheim, the Secretary-General of the United Nations, is no doubt hoping that by the time the next report on the subject appears, his own continued tenure of his important post will have been confirmed.

The tale of the yellow rain over South-East Asia is far too tortuous for anybody's comfort. The allegation that aerial sprays containing unusual amounts of mycotoxins have recently been sprayed over South-East Asia was first raised at the end of last year but was brought more firmly to public attention some weeks ago by the United States Secretary of State, Mr Alexander Haig, in a speech in Berlin. The subsequent tour of European capitals by a group of Mr Haig's choosing was notable partly for the anonymity of its members but chiefly for the vagueness of the extra evidence it was able to produce (see *Nature* 22 October, p.598). The group left those who heard what it had to say with the firm impression that it would have been happier if there had been more substance in what it said.

Unhappily, the passage of time is death for innuendo. Mr Haig charged that the Soviet Union sprayed mycotoxins from the genus *Fusarium* on innocent people. In was never self-evident why such a stratagem should be followed, even by supposedly malevolent people. Why not anthrax, for example? The classical disadvantage of biological weapons is, after all, well-known to be their slowness. Furthermore, even though further samples of soil containing mycotoxins have been produced, they may be there naturally, and it is premature to claim it as proof that these chemical agents were used. What the US government should do is to publish the data it has to hand in the scientific literature and let others judge for themselves what should be made of it. Jumping the gun is no way to world peace.

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Fusion chief resigns over budget cuts

Mirror devices and tokamaks in conflict

Washington

The head of fusion research programmes in the United States Department of Energy (DoE) has resigned in protest at the way in which the Reagan Administration is proposing to allocate research funds between competing magnetic fusion projects.

His decision has apparently been provoked by the decision of the Office of Management and Budget (OMB), in drawing up the recommendation that President Reagan will present to Congress next January, to shift $25 million from research into mirror devices being studied at the Lawrence Livermore National Laboratory in California to the Tokamak Fusion Test Reactor (TFTR) at the Princeton Plasma Physics Laboratory in New Jersey.

The most immediate impact, according to Dr Edwin Kintner, who has been head of DoE's research programme since 1977, and who last week submitted his resignation as associate director of the Office of Energy Research, will be to delay by about three years completion of the expanded Mirror Fusion Test Facility (known as MFTF-B) which many consider to be the principal competitor to the tokamak.

But the Administration's decision has broader implications, for it effectively undermines a strategy which has been developed by the department under Dr Kintner to diversify magnetic fusion research away from too great a dependence on the success of the Princeton work.

Support for such diversification was first endorsed by the Administration after a report prepared for DoE by an *ad hoc* committee under the chairmanship of Dr John S. Foster of TRW Inc. in 1978. This pointed out that although the Princeton tokamak programme was achieving impressive scientific results, many engineering problems remained before a viable power reactor could be built.

Concentrating purely on the tokamak, it said, constituted an "unnecessarily high risk". It suggested that the United States should "pursue fusion on a broad front", primarily into mirror devices but also into alternative designs.

After careful review, DoE selected two of these designs for increased support. One was the reversed field pinch, a combination of the tokamak and mirror designs. The other was the Elmo Bumpy Torus (EBT), under development at the Oak Ridge National Laboratory in Tennessee (see *Nature* 1 May, p.3).

Mirror research was given further support by the successful development at the Lawrence Livermore Laboratory of tandem mirrors to plug the ends of a solenoid-containing plasma. As a result, it was decided last year to expand the first MFTF into a tandem mirror machine, MFTF-B, scheduled for completion in about four years' time and to perform at temperatures and confinement times comparable to the performance of the tokamak.

This strategy is now coming apart under the stringent budgetary pressures being applied by the Reagan Administration. The first potential casualty is likely to be the EBT "proof-of-principle" machine (EBT-P), proposed for construction by the Carter Administration under contract with McDonnell Douglas, but cut from DoE's budget by Mr Reagan in March. Although Congress has reinstated the money in an appropriations bill now sitting on the President's desk, which would provide $14 million for EBT-P in the current year, it seems unlikely that he will sign it.

Overall, the 1982 budget for magnetic fusion does not look too bad. Although this was reduced in March from the $506 million proposed by President Carter to $456 million — partly by eliminating funds for a centre for magnetic fusion engineering which DoE had previously agreed to build under congressional pressure to move faster on magnetic fusion in general — the latter figure is still sufficient to maintain a cost-of-living increase for the current financial year.

As with other federal agencies, however, it is the 1983 budget that is giving the greatest cause for concern in Washington as the Administration seeks further substantial cuts in public spending to reduce the federal deficit while increasing the military budget.

OMB's decision to shift support from the mirror programme to the TFTR "is a precedent-setting decision taken without an adequate hearing or technical discussion", Dr Kintner said last week, adding that he had been unable to persuade DoE to appeal and that it was going "to change the whole policy direction of magnetic fusion research for the future".

"For the first time we had a policy which made sense for fusion. It did not argue that we should start competing with other forms of nuclear energy by a definite date, but said we should first meet the short-term objective of knowing whether fusion is practical as an energy source. I worked for five years to create cooperative relationships and research strategies which made sense. That is all dead."

While the strategy of diversified research support has been pursued within DoE, however, it has come under some criticism from the nuclear industry and its congressional supporters who would like to see work proceed on an experimental tokamak reactor as quickly as possible and feel that the DoE strategy may have been overly cautious. **David Dickson**

Cline stripped of research grants

Washington

In a move clearly intended to warn other scientists not to ignore restrictions laid down by the federal government, Dr Martin Cline of the University of California in Los Angeles (UCLA) has been stripped of one of four research grants he receives from the National Institutes of Health (NIH) because he carried out unauthorized experiments on human patients that involved the use of recombinant DNA techniques.

The incident first came to light last autumn, when it was revealed that, while treating patients in Israel and Italy suffering from β-thalassaemia, Dr Cline used bone marrow cells whose genetic material had been altered by recombinant DNA techniques, even though he had previously told both his university and the two hospitals involved that he would not do so.

Earlier this year, an investigatory committee established by NIH suggested various conditions that should be applied to future grants applications from Dr Cline, who had already resigned as chief of the division of haematology/oncology at UCLA, while retaining his university post as professor of oncology.

In the light of their conclusion that he had violated both federal regulations for the protection of human subjects and NIH guidelines for use of recombinant DNA technology, the members of the committee recommended — and the NIH director (then Dr Donald Fredrickson) accepted — that special prior approval be required for all future grant applications from him for research with either human subjects or recombinant DNA (*Nature* 4 June, p.369).

It was left to the advisory councils of various NIH institutes funding his research to decide what to do about existing grants. Two of them, the National Cancer Institute and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, have taken no direct action.

The advisory council to the National Heart, Lung and Blood Institute (NHLBI), however, decided on a harsher penalty than merely impressing conditions on future applications. It recommended that Dr Cline's current grant from the institute be terminated on 31 March 1982, the end of its first year of support.

The grant, a total of $244,000, was to have been spread over a period of three years, and covered research into the effec-

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tiveness of the insertion of human β-globin genes into mouse haematopoietic cells. A condition of this grant was that no work on human subjects should be involved.

In the case of the National Cancer Institute, two grants for which Dr Cline was the principal investigator were reviewed. The first, for research into normal and malignant haematopoietic cell replication, has been allowed to run until its expiry date at the end of next May, and a renewal application is now under consideration.

On the second grant, part of a four-year project in medical oncology with a total cost of $3.3 million, the National Cancer Advisory Board has suggested that the grant be continued until 28 February 1982. A renewal grant application has already been submitted by UCLA, with Dr David Golde named to replace Dr Cline as the principal investigator.

Following the decision by Dr Tom Malone (acting director of NIH) to accept the recommendations of the three advisory boards, Dr Cline has pointed out that the termination of the NHLBI grant eliminates only about 20 per cent of his research support. He has also said that he has not decided whether or not to appeal against the decision. In a letter to NIH written in September, however, Dr Cline strongly criticizes the 14-month delay by the UCLA Human Subject Protection Committee in giving a definitive response to his request for permission to carry out experiments.

One sanction recommended by the NHLBI advisory council which was not endorsed by NIH was that Dr Cline be asked to provide assurances that he will not engage in human experimentation involving recombinant DNA for a three-year period. A memorandum from NIH associate director of extramural research, Dr William Raub, says that he does not believe it appropriate for NIH to impose such a sanction, "nor do I believe we have authority to do so". **David Dickson**

ARC biotechnology

Cottage industry?

The pips may squeak in British research, but biotechnology forges on. The UK Agricultural Research Council (ARC), whose budget shrank 3 per cent in real terms between 1979–80 and 1980–81, has actually been able to announce the setting up of a new research centre. Admittedly, it is in a portable home (a Portakabin), but it will house twelve researchers and provide a focus for ARC work on monoclonal antibodies.

Costing around £100,000 for the Portakabin and equipment, and £100,000 a year to run when it gets going in April next year, the centre will have two resident researchers, four technical staff, and — on average — six visitors from other ARC laboratories.

The goal is to produce a centre of expertise in the handling and creation of hybridomas — which some ARC virologists have found to be tricky things to culture. The resident researchers at the new centre will work on their own projects (for example, suggest ARC officials, on creating hybridomas of cow, pig and sheep cell lines) and assist visitors with their own problems.

The first applications will be to the creation of specific antibodies to viral strains, such as the varieties of calf enteritis virus, to help research and — perhaps — create vaccines where these would be commercially useful. To this end the unit will also have production facilities large enough to conduct commercial trials.

Who will profit from this ARC commercialism, however, is a moot point. By law the council must pass patent rights to the National Research Development Corporation, now part of the British Technology Group which includes the National Enterprise Board. Unlike the Medical Research Council, ARC has no direct agreement with Celltech, the company created by the National Enterprise Board a year ago to exploit biotechnical developments in British research establishments and universities (and to pursue its own research); but, says Celltech, it would expect the new British Technology Group to consider them as potential developers of any ARC product. The British Technology Group, however, would be free to approach any company it wished — and that might be a new British Technology Group company specializing in agriculture.

ARC recently suggested that the British Technology Group should set up a kind of agricultural parallel to Celltech — in which, no doubt, the ARC would like to have the same exclusive rights and potential earnings as the Medical Research Council has in Celltech — and this idea is still being considered. The British Technology Group will not reveal what stage negotiations have reached, but the ARC do not seem particularly optimistic.

Celltech itself is certainly interested in veterinary applications of hybridomas, but the company does not want to be thought of as specializing only in monoclonal antibodies. Its first and so far only product is a monoclonal antibody against interferon, but Celltech's research and development is now evenly divided between monoclonals and recombinant DNA. Celltech also sees no immediate likelihood of involving itself in the genetic manipulation of plants, unlike the ARC, which has the area very much in mind.

Meanwhile ARC was last week still awaiting official confirmation of the European Council of Ministers agreement three weeks ago to go ahead with the European Commission's biotechnology research programme, which will specialize in agricultural applications. ARC has been closely involved with the definition of the programme, and might hope to win up to £100,000 a year in grants to supplement its own £1 million annual spending in biotechnology — but only if it has the chance to appoint a strong scientific team to the Advisory Committee on Programme Management. This Brussels committee will ultimately examine research proposals within the programme and — by advising the Commission — effectively hand out the cash. Unfortunately ARC is at a far remove from the Department of Industry, which is in touch with Brussels on this matter, and there are fears that the council may not be approached in time for Britain to get strong representation on the committee.

Robert Walgate

Soviet universities

Research needed

The Soviet Union must invest more in university science, according to Academician Ivan F. Obraztsov, Minister of Higher and Secondary Specialized Education of the Russian Republic. Writing in *Pravda,* he claimed that unless academic research is given priority, it will be difficult to train "good cadres" in the new directions needed for the Soviet economy to develop.

Obraztsov said that Soviet universities and higher educational institutions at present receive no capital funds specifically earmarked for science, although they carry out research worth more than 1,200 million roubles each year. Funds for scientific equipment and research materials are not forthcoming, and computers and similar sophisticated equipment appear far later in the universities than in institutes and laboratories run by specialized ministries.

The lack of funds seems to be especially serious in the engineering faculties. Although Soviet industry is committed to a policy of extensive automation, which is made more urgent by the Soviet Union's falling birth rate, the training of future engineers needs to undergo a "major restructuring". Obraztsov implied that means of familiarizing students with automated design systems, control systems, production lines and man–machine complexes simply do not exist.

The emphasis on engineers reflects a trend in the Soviet press. After a long "press debate" last year on why Soviet public opinion gives more prestige to the pure than to the applied sciences, the weekly *Literaturnaya Gazeta* last month published a round table discussion on the role, status and career prospects of Soviet engineers.

One complaint was that since 1948, engineers' salaries had risen by an average of 20 per cent in real terms, while those of "ordinary" workers had doubled. The participants also noted that young people seem reluctant to apply to engineering colleges. Many of the most prestigious higher technical schools, such as the Leningrad Mining Institute, no longer set competitive entrance examinations, while

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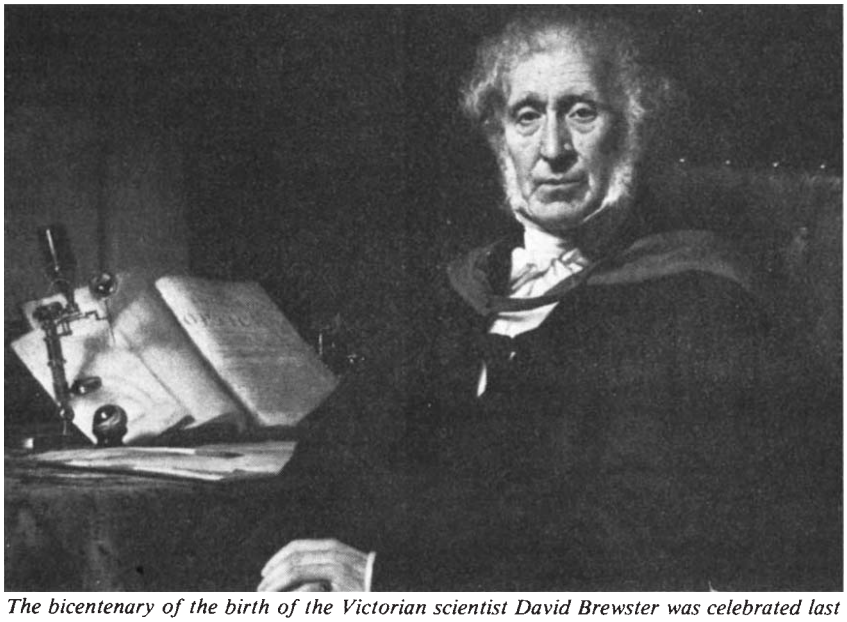

The bicentenary of the birth of the Victorian scientist David Brewster was celebrated last week in a one-day symposium held at the Royal Scottish Museum in Edinburgh. Brewster's main claim to fame was his invention of the kaleidoscope, but he also made important contributions to work on the polarization of light. He later became Principal of the Universities of both St Andrews and Edinburgh, and was a major force in the establishment of the British Association for the Advancement of Science.

in a certain "fashionable" mathematics and mechanics faculty, the ratio of applicants to places was only 1.2 compared with 19.0 in a similar trade institute.

The impression that applied projects now take precedence was reinforced by last month's award of state prizes for science and technology. The projects honoured included work on urban water supplies from underground sources, the enrichment of desert pastures, bone transplants for diseases of the jaw and the future development of the fuel and mineral deposits of the Kansk-Achinsk basin. Even an apparently theoretical study of high temperature plasma was, according to Professor Nikolai Arzhanikov, the secretary of the awarding committee, "a major contribution" to the development of thermonuclear power.

The yearly stocktaking of Soviet life and progress which follows the Revolution Day parade included extensive press discussions of how to accelerate the implementation of research results in Soviet industry. Dr Boris Paton, president of the Ukrainian Academy of Sciences, suggested that the concept of "implementation" might profitably be deemphasized and the stress placed on "cooperation" between science and industry. The distinction is not trivial; enterprises which have an active role in developing new technologies, rather than simply "implementing" the results produced by some research institute, will be eligible to share in major Soviet prizes.

Whatever the form of the link between science and industry, however, it ultimately depends on an appropriate supply of scientists and technologists. During the past decade, science planners have concentrated on linking universities and technical institutes into regional "science centres" whose research, it has been urged, should serve the needs of local industry. Obraztsov's complaints of the plight of universities suggests that too little attention has been paid to their major function as teaching and training establishments. **Vera Rich**

Clean air legislation

Forces massing

Washington

Indecision within the Reagan Administration about how to reform the Clean Air Act is playing directly into the hands of environmentalist groups and their congressional supporters who feel that the act should be strengthened rather than weakened. It is also upsetting industry groups who had looked to the new Administration to reduce the burden of environmental and health regulations.

The current law was passed in 1970, and must be renewed by the Senate before the end of the year. The debate on revising the law is regarded as the first major test of President Reagan's determination to pursue his election promises of regulatory reform.

Even some members of the Carter Administration have argued the need for significant changes in the act. Last week the Brookings Institution in Washington published a paper by economist Lester B. Lave and Dr Gilbert Omenn, previously associate director for health sciences in the Office of Science and Technology Policy, which is sharply critical of current air quality legislation.

Claiming that the main reason the nation's air has become cleaner in recent years has been the switch to cleaner energy-producing fuels, Dr Lave and Dr Omenn propose that the act should consider individual differences in susceptibility, as well as the availability of personal preventative health measures, when determining the acceptable "remaining risk" resulting from the various levels of proposed controls.

Industry itself is pushing for even greater changes. Public utilities, for example, are strongly resisting efforts to write into the act significant extra controls on emissions from power stations aimed at reducing the problems of so-called "acid rain" formed when sulphur dioxide reacts with atmospheric moisture.

How much is eventually changed, however, depends largely on the results of debates within the Senate Environment and Public Works Committee. And so far the lack of a clear battle plan from the Administration has left the initiative with those fighting to maintain the status quo.

The level of permissible emissions from motor vehicles has been one of the most contentious parts of the 1970 legislation. At the time, Congress set tight numerical limits on automobile and other vehicle emissions, with a strict timetable for their implementation, although under pressure from the automobile industry the deadlines have been gradually put back.

In their report for the Brookings Institution, Dr Lave and Dr Omenn argue that Congress should not be involved in setting detailed regulations for motor vehicle emissions because of the unnecessary rigidity that results. Instead, they say, Congress should delegate more, although still retaining the oversight role.

The Senate committee does not agree. For example, Environmental Protection Agency (EPA) assistant administrator Kathleen Bennett told the committee that, because of the expense involved, the Administration proposed to lower from 90 to 60 per cent the reduction in truck hydrocarbon and carbon monoxide emission required by 1984. But Senator Gary Hart replied that the figure of 60 per cent had been "plucked from the air" and argued that EPA should not be making economic decisions on public health issues.

Within the Administration, much of the blame for the current disarray in EPA's strategy is being placed on Administrator Mrs Ann Gorsuch, who announced in August that she would not be seeking major changes in the act, but was rather pushing for the inclusion of several basic principles on which future regulation should be based (*Nature* 13 August, p.574). The result has been serious concern in several industries that the Administration is failing to fulfil its election promises. At the same time there is relief among Republican congressmen who already feel the worsening economic situation is jeopardizing their chances of re-election next year. **David Dickson**

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Commercial rocketry

One for Ariane

Ariane, the European satellite launcher, has at last won a commercial place in the United States market. Last week, Arianespace, the French-based company which manages commercial exploitation of the European rocket, signed a firm $50 million contract with the US General Telephone & Electronics (GTE) Satellite Corporation to launch two telecommunications satellites in 1984. The order, the first that Arianespace has won from an American customer, seems to confirm at least some of the fears of US space officials that Ariane could take business from the National Aeronautics and Space Administration, which until now has enjoyed a virtual monopoly outside the Eastern bloc for launching heavy satellites.

Ariane's chief attraction for US customers, who enjoy a 25 per cent discount, is its relatively low cost. At about $25–$30 million per satellite, an Ariane launch is 15–20 per cent less than a launch on a Thor-Delta rocket. Delays and uncertainties over the space shuttle have also made Ariane more competitive. Arianespace is optimistic that last week's deal will be followed by others.

Arianespace officials seem pleased with their own marketing efforts and those of the Grumman Corporation, their US agents. Twenty-one satellites from twelve customers, mainly European, have so far been committed for launch on sixteen Ariane flights. Three firm contracts, including that with the GTE Satellite Corporation, have been signed and sealed since the third successful Ariane test flight last July. In addition, ten potential customers hold reservations for launching sixteen satellites on nine Ariane flights.

The order books are as healthy as can be expected after only three out of four test flights, according to Arianespace. Their future health, however, will depend on the fortunes of the fourth test flight scheduled for 18 December. **Judy Redfearn**

European cooperation

COST in limbo

Brussels

The celebrations last week marking the tenth anniversary of COST, an organization pursuing "European Cooperation in the Field of Scientific and Technical Research", were appropriately low-key. Indeed, the role of COST itself has become so unobtrusive that its officials fear that its future is endangered by widespread ignorance of its purpose and even of its existence.

The chairman of the COST Senior Officials Committee, Johan Martin-Löf, speaking to the small group of senior officials and diplomats which had gathered to celebrate the anniversary, did his best to emphasize COST's solid achievements: that 35 different research projects had begun in the past decade, ten of them had been completed, and that this had involved ten EEC member states and nine other West European countries, all for a small extra outlay.

Part of COST's problem is that it is not a conventional international organization but a club which invites member countries to share the results of usually public research. Once a project has been agreed by the intricate committee structure, individual member countries select the aspects they wish to work on and by financing only its own share of a project, each participant country has the right to the results of the entire research effort.

COST's vague status has complicated the administrative problems of co-ordinating the research programmes of 19 countries. A small secretariat in Brussels is its only direct physical manifestation. A major obstacle for those taking part in COST projects is the financing of travel. And Martin-Löf admits that "the time it takes to handle even relatively simple matters is often excessive". A further problem is the lack of money for the administration of projects once launched.

Suggestions for new projects have to come from the member states themselves and the most dynamic field for new projects seems to be in telecommunications, transport and materials science. Last week, a memorandum of understanding was signed for the COST project on high-temperature materials for power plants. **Jasper Becker**

Harsh sentence for Paritskii

A Khar'kov court has sentenced Aleksandr Paritskii, a 43-year-old specialist in electronics, to three years in a labour camp. The sentence was the maximum possible for his formal offence — disseminating anti-Soviet propaganda. Its severity may be due to one of Paritskii's activities which was not mentioned in the formal charge — the organization of a weekend "university" for young refusniks and the children of Jewish activists.

Exclusion from higher education is one of the regular sanctions imposed on those Jews caught in the limbo between applying for an emigration visa and actually being allowed to leave the country, and *a fortiori* on those formally refused permission to leave. Although since 1973 an increasing number of unofficial seminars have been organized for refusniks excluded from employment in the academic professions, until recently nothing formal had been done at undergraduate level.

In autumn 1980, however, Paritskii and a handful of fellow activists in Khar'kov founded their "refusniks' university" with 25 students. Structured courses were held every weekend, throughout the academic year. Mounting harassment of Paritskii, followed by his arrest, has made it impossible for the university to reconvene this year.

The absence of the university from Paritskii's charge-sheet is interesting. Paritskii's colleagues from the "university" were not even called in for interrogation in connection with his trial. This may well mean that the authorities fear that an open attack on the "university" may simply result in a similar initiative springing up elsewhere and hope that with Paritskii gone, the "university" will simply disappear. Certainly the authorities seem to be interested in undermining his academic reputation. Earlier this year, he was one of three refusnik scientists who, in a move unprecedented in the Soviet Union, were deprived of their "Candidate" (PhD) degrees because of their political views. **Vera Rich**

Asian ecology

Loss of balance

Bangalore

The increasing demand for wood for fuel in Nepal and Thailand is threatening the ecological balance of a large part of South Asia. In Nepal, deforestation is so rapid that it has been estimated that the country will be without forest by the year 2000.

The lack of tree cover is already causing two major problems. First, the once fertile valley lands are losing their ability to assimilate rainwater, causing a decline in vegetation, fast-spreading aridity and bird migration. Second, an estimated 240 million cubic metres of top soil is being carried into streams and rivers flowing into India and Bangladesh.

It was not until 1976 that the Nepalese government acknowledged the magnitude of the danger. It has now launched a 400 million rupee project, to be financed by the International Development Agency and other organizations, which aims to convert nearly 50,000 hectares of land into community forests over the next five years to meet the growing need for firewood.

In Thailand the government is also undertaking a replanting scheme concentrating on high-yielding rubber trees. The old unproductive rubber trees are to be used as construction materials in a plan which should, says the Thai Agriculture Ministry, bring in around $60 million. Trials have shown that trees can be sprayed with chemicals and conserved at plantation sites for up to two years — at present more than 80 per cent of old rubber trees are felled and burned in a haphazard manner. Agricultural scientists have also examined the possibility of using the old trees for pulp. **B. Radhakrishna Rao**

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Theory of evolution

Huxley speaks up

Sir Andrew Huxley, his grandfather's grandson, startled the Anniversary Meeting of the Royal Society this week with a stout defence of the theory of evolution. Traditionally, presidents have used these occasions for making gentle complaints about the British government's handling of science and education. But Huxley, starting with Sir Edmund Leach's statement at the British Association meeting this year (see *Nature* 3 September, p.19) that many of Wilberforce's nineteenth century criticisms of Darwin were now accepted, said "here is a Huxley answering the challenge".

The correspondence in the past twelve months about cladistics and the British Museum (Natural History) still seems to rankle. In defence of cladistics, Huxley said that even the insistence of "transformed cladists" that taxonomic classifications should be made without reference to "necessarily conjectural" ideas about evolutionary processes and selective pressures "is in no sense a denial of evolution".

Huxley said that his reaction to the correspondence in *Nature* had been to wonder whether he should "take up the cudgels on Darwin's behalf", but that he had been "mollified" by a "very sensible editorial article" in *Nature* (30 July, p.395). His concern now was to counter the "ripples [which] continue to spread".

In passing, Huxley said that the calculations of Hoyle and Wickramasinghe (see *Nature* 12 November, p.105) suggesting that there could not have been enough time since the origin of the Universe for life to have evolved could be "dismissed quickly". He said that the characteristic large number used by the authors ($10^{40,000}$) represents the chance that 2,000 enzyme molecules would be formed simultaneously "*on a single specified occasion*". "Since we know neither the nature of the hypothetical self-replicating system nor the composition of the primaeval soup", the conclusion is, however, "wildly uncertain".

It is unlikely that Hoyle and Wickramasinghe will accept their dismissal on these grounds. Their arguments are based on the "information content" of the evolved genome, and on estimates of the rate at which information content might plausibly accumulate.

Huxley in defence of evolution

On the doctrine of Gould and Eldridge of punctuated equilibria, Huxley said that the debate lies within the Darwinian framework but that its authors have exaggerated the suddenness with which new stable species emerge. In any case, he said, Darwin's own guess that it would take ten or a hundred thousand generations for two "well-formed" species to emerge from a single species is not inconsistent with the doctrine of punctuated equilibria, while later editions of *The Origin of Species*, like the writings of neo-Darwinians such as G.G. Simpson, contain statements which are "exactly equivalent" to punctuated equilibria. In the circumstances, Huxley said, he could not understand how punctuated equilibria could be regarded as contrary either to Darwin's own ideas or to those of neo-Darwinism.

On Sir Edmund Leach's echo of Wilberforce's complaint that the fossil record lacks evidence of the "missing links" between major stable groups of species, Huxley replied with a list of "missing links" whose discovery "has gone on at an increasing rate since before the publication of *The Origin of Species* to the present day", such as *Archaeopteryx* (1861), the fossil horses and hominids more ancient than the Neanderthals.

Huxley went on to dismiss other complaints made by Wilberforce and Owen a century ago, chiding anti-evolutionists for overlooking the "positive indications" from biochemistry, which have demonstrated the "ubiquity of the main metabolic pathways" and of Mendelian inheritance — "precisely what Darwin needed".

Likening the present status of evolution to that of biochemistry fifty years ago, Huxley listed the remaining "gaps" that seem to him important — the absence of evidence for the origin of the major groups of animals in the Palaeozoic, the inheritance of interspecific sterility, the role of genetic drift, the origin of life and the existence of consciousness — "too often swept under the carpet".

University problems

Among the points in the secular part of Sir Andrew Huxley's anniversary address on Monday were the following:

- The dual support system for academic research, "a shadow of what it used to be", is in danger of being totally obliterated.
- Although the British government had agreed in its expenditure white paper in March to protect basic research, research councils are having to spend more on making good deficiencies of university budgets and are thus less able to support new research projects.
- There is a danger that universities will respond to this summer's budget cuts by further reductions of support for their own research.
- If a university "shows that it is moving in the right direction", the British government should be prepared to reduce the speed or the severity with which cuts are required.
- On the increased fees for overseas students, "I wish to add my voice to the many who have expressed dismay at this drop in our contribution to development, and at the loss of goodwill towards Britain that this policy will entail in the long run".

The society's annual report, also published this week, records that its own subvention from the British government increased to £4.2 million in the year just ended, from £3.7 million the previous year. General administration cost £564,000 — the rest was spent on support for people, international exchanges, grants for research and on international subscriptions.

Rhesus monkeys die

The University of Birmingham is being forced by financial and legal circumstances to kill most of its once thriving monkey colony.

Unable to raise the £250,000 needed to bring its monkey housing up to present safety standards, the university has for the past two years been exploring the possibility of dispersing all the animals elsewhere. Although there is no shortage of willing recipients, the university has finally decided that the probable legal risks in dispersing its adult monkeys are too great to delay any longer what they see as the almost inevitable slaughter of the animals.

The legal difficulties arise because almost all the adult rhesus monkeys have been used for experimental purposes under Certificate B of the Prevention of Cruelty to Animals Act which demands that animals be killed at the end of an experiment. Many experiments on the Birmingham monkeys were in midstream when they were stopped by the university pending modernization of the housing.

Since the experiments were interrupted rather than finished, it has been argued that the monkeys could be transferred elsewhere and experimentation continued. But the university, in consultation with the Home Office, has decided that it would be too risky to test that interpretation of the law. **Peter Newmark**

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CORRESPONDENCE

Hoyle on life

SIR — Surely there is some mistake in your summary of Hoyle's contention that the Universe has not been here long enough to permit the evolution of life forms of the complexity found on this planet (*Nature* 12 November, p.105).

It is true that the probability of chance assembly of the system of genes upon which our life system depends is very low. However, it is not this particular probability which is relevant, but rather the result of multiplying this admittedly small number with the very large number representing the total number of gene systems which could also be the basis for complex life forms.

Any particular gene possesses its enzymatic properties as the result of its configuration in some quite small part of its total chain length. Although the chance of random assembly of the total chain, specified at every point, is small, the chance of assembly of a gene specified over a small fraction of its length is obviously much larger.

If you modify your account of Hoyle's arguments along these lines, I suggest you will conclude that we are all here after all.

A.E. ROUT
British National Oil Corporation (Development) Limited, Glasgow, UK

Postdocs in Canada

SIR — I would like to pass on some news that I have just obtained with regard to the status of foreign postdoctoral fellows working in Canada. The Canadian Immigration Regulations require that before a foreigner can be offered employment, the position must be advertised through Canada Employment Centres, and suitably qualified nationals must be given preference. This regulation does *not* apply to postdoctoral fellows, but Canadian officials both in London, England and in Toronto assured my wife and myself that it did apply to their spouses. When we came to Canada last year my wife was unable to find a job, and since postdocs in biological sciences are not well paid (see *Nature* 11 June and 8 October), it became necessary for my wife to return to England to work.

This seemed an unfair system, so I brought it to the attention of Lloyd Axworthy, the Canadian Minister of Employment and Immigration. I have received a reply from him to the effect that since such restrictions do not apply to Canadian postdocs going to work in Britain, Canada is prepared, in this case, to reciprocate. He is instructing all Canadian immigration officials that this is now the case. So in the future, married postdocs will be able to move between both countries without facing financial hardship. However, it is implied that this relaxation does not apply to individuals from countries which do not already show hospitality to Canadians. I am impressed by the fact that the experience of an individual can successfully modify governmental regulations, but surely it is opportune, and for the mutual benefit of all concerned, for all countries which wish to foster academic exchange to take steps to relax immigration rules for the families of postdoctoral fellows.

DYLAN EDWARDS
The University of Western Ontario, London, Canada

Open relations

SIR — Your article about the agreement between this hospital and the international chemical company Hoechst AG (*Nature* 5 November, p.5) states that the Government Accounting Office forced open the contract heretofore kept from Congressman Albert Gore. In fact, Massachusetts General Hospital gave the document to Congressman Gore on 21 July 1981.

What we did in October was to make the contract available to the general public from whom we have had a number of requests. In fact, we know of no other instance in which such a contract has been made available to the public. We have taken this course because we are presenting the agreement to our external Scientific Advisory Committee this month. The committee's topic, scheduled many months ago, is scientific relations with industry.

JOSEPH B. MARTIN
Massachusetts General Hospital, Boston, Massachusetts, USA.

Life out there

SIR — It was interesting to read in juxtaposition Dr Zuckerman's review of the extraterrestrial issue and Professor O'Neill's review of Rood and Trefill's new book on the same subject (*Nature* 5 November, pp.10, 25). One is struck again by the fascination — not to say fixation — that earlier and "young revolutionary" students of this issue have had with technology as an index of "advanced" civilization. The idea that other planets may have chosen to focus on spiritual rather than technical development dates back to at least the eighteenth century polymath Swedenborg. As is often noted nowadays, the validity of technology — rather than, say, spirituality — as an index of cultural achievement is less than clear even for our own globe. It seems premature to disregard the Drake (or Green Bank) equation simply due to lack of verification of its technology/colonization components. What is called for instead, perhaps, is greater conceptual bandwidth in its formulation.

KURT SIMONS
Wills Eye Hospital, Philadelphia, Pennsylvania, USA

Leave links open

SIR — Dorothy Hirsch, Executive Director of the Committee for Concerned Scientists, ended her eloquent letter (*Nature* 13 August, p.578) about the plight of Soviet scientists with the charge to us that "We cannot afford to become hardened to the plight of oppressed colleagues who have much to contribute to a vital international science". *Time* (28 September) reports that exiled Soviet writers, despite enormous grievances against the Soviet government, have pleaded that free world publishers should not boycott the Moscow International Book Fair which means so much to the ordinary Russian citizen as well as the Russian literati. I have just returned from an international medical/scientific congress in Moscow, and wish to make a similar appeal to Western scientific groups resolved to boycott all scientific interchanges hosted by the Soviets.

At that congress I concluded my scientific presentation with a slide listing a dozen of the Soviet scientists sentenced to "internal exile" (imprisonment) for their dissident views, and stated simply that my presentation was dedicated to "these fellow scientists in the sincere hope thay they may be free again some day *soon* to join with their colleagues from other nations in open scientific interchanges such as this one". It is still tearful for me to recall the response of the stony-faced Russian scientists in the audience to that statement. Almost in unison, they lowered their eyes, nodded very slightly, and a whispered chorus of "Da! Da! Da!" ("Yes! Yes! Yes!") filled that Moscow meeting room.

I appeal to individuals and scientific societies that have decided to boycott Soviet-hosted scientific interchanges as a protest that they reconsider their decision. Boycotts do little to embarrass or punish the Soviet government for its repressive and reprehensible policies towards dissident scientists (writers, intellectuals, etc.); attendance at these scientific interchanges as representatives of free world countries may do much to assure the Russian scientific/academic community of our awareness of their plight, and of our deep concern and sympathy for them. It lets them know of our resolve not to cut them off from new information from the outside world, but to share with them our scientific findings, our ideas, our literature, which they, like us, should not be denied. They are obviously not optimistic that things will change in the near future. Let us at least continue to help them endure what they must so that they can continue with their careers, with their scientific research which they love as much as we do.

LEE FRANK
Department of Medicine, University of Miami, School of Medicine, Miami, Florida, USA

Not cricket either

SIR — Certainly the City of New York's Yankees deserve numerous boos and catcalls for their performance in the World Series. Just as certainly, your publication deserves similar treatment for printing a column ("Shame on New York", *Nature* 5 November, p.2) demonstrating both little understanding of the subject and a shameful neglect of facts evident to all but the most illiterate American youth.

You state "Part of the reason why the Yankees lost is that they were not as skilled at catching and throwing the ball as were the Dodgers", a glaring misstatement in light of the Dodgers' second baseman's infamous accumulation of six World Series records for "errors committed" (literally: failure to catch and throw the ball). In addition, claiming that there was "little to choose between the batting performance of the two teams" ignores the dismal failure of one of the Yankees' most depended-upon batters (Dave Winfield, playing under a multi-million dollar contract) to connect for more than one hit in twenty-two at bats during the series.

Concerns for propriety and my own temperament forbid me to further chronicle and refute the errors contained within the column. Allow me to end my tirade by pointing out your insensitivity in measuring the speed of a baseball in units of "metres per second" thereby violating the worshipful reputation of baseball as "a game of inches".

PETER SYKA
The Salk Institute, San Diego, California, USA

NEWS AND VIEWS

Consider the incompleteness of the geological record

from Tjeerd H. van Andel

FOR more than a century geologists have felt comfortable with the notion that geological processes, although very slow, are also steady and so capable of moulding the Earth given enough time. The steady drip hollows the stone. The idea of steadiness came, at least in part, in reaction to the catastrophist view of the early 19th century which saw the history of the Earth as punctuated by brief but violent upheavals separated by long times of unchanging tranquillity. The slowness was suggested by the difficulty of observing many geological processes at work, and was enhanced by the growing awareness of the great length of time that was available. The rise of a continent may not be visible in a lifetime, nor the removal of a mountain by erosion, but the 4.5 billion years of Earth history provide enough time for all recorded events.

In recent years we have learned that many, perhaps most, geological processes are more rapid than once believed. The Atlantic Ocean widens at a rate of 20 km per million years, the rise of sea level at the end of the last Glacial locally pushed the coast inwards at a rate of 3 km per century. Every year the Mississippi delta adds 50m of land to the state of Louisiana and, if left undisturbed, the equatorial ocean would fill with sediment in a few hundred million years. If these rates are typical, do we need all of the available time to account for the events recorded by the rocks?

The answer is clearly negative. Studies of modern depositional environments taught us the rates of deposition typical for each. We may apply these rates to the deposits left behind by their equivalents of the past if we make allowance for such matters as compaction due to overburden and time. For years I have been intrigued by the result of such estimates. In Wyoming, a sequence of early Cretaceous sandstones and shales closely resembles the coastal sediments of the present Gulf of Mexico. Applying the appropriate rates of deposition we find that a mere 100,000 years would suffice to produce the entire sequence. Yet the stratigraphical interval occupied by the deposits is quite firmly known to encompass about 6 million years. We may repeat the experiment elsewhere; invariably we find that the rock record requires only a small fraction, usually 1 to 10 per cent, of the available time, even if we take account of all possible breaks in the sequence. Evidently deposition, unlike work in Murphy's law, does not expand to fill the time available. This might in principle be expected but the universality and especially the magnitude of the shortfall are startling.

This fascinating observation has attracted the interest of a few geologists but only in passing. Recently, however, it has inspired Peter M. Saddler to examine the issue in depth (*Journal of Geology* **89**; 569, 1981). If it is indeed true that ancient sediments represent less time than available, the *accumulation rate* obtained by dividing the thickness of a deposit by the length of the interval it occupies ought to be smaller than the deposition rate measured directly in the equivalent modern environment. This, indeed, turns out to be the case and the fractions of time not represented by deposits are very large. Even more interesting, the massive amount of data put together by Saddler shows that the accumulation rates (thickness/available time) are inversely proportional to the length of the interval over which they are estimated. This inverse relationship holds true for all kinds of environments, even though the precise form varies.

Because one can resolve very short intervals only in young deposits while very long ones carry us far into the past, one might surmise that what we are observing is a progressive increase in the rate of deposition towards the present. This assumption, unattractive for several good geological reasons as well, is incorrect because the inverse correlation exists between the accumulation rate (or the thickness of the preserved sediment) and the duration of the observed interval, but not its age. Thus it appears that indeed the geological record is exceedingly incomplete, and that the incompleteness is greater the shorter the time span at which we look.

Tjeerd H. van Andel is professor of oceanography in the Geology and Geophysics Departments at Stanford University, California.

It does not startle us that the geological record is damaged by gaps; we have known that for a long time. Nor is it surprising that the amount of time represented by breaks is significant. Some time ago, before the deep-sea drilling programme of the *Glomar Challenger,* it was widely believed that the sediment record in the deep ocean should be very complete because the environment is quiet and is the ultimate resting place of all debris. This turned out to be far from true. In the South Atlantic, for example, barely half of the history of the last 125 Myr is recorded in sediment. It is no better in other oceans and surely worse for shallow marine and continental environments. Even so, the level of incompleteness with which we are concerned in this paper is far in excess of what has been observed or might reasonably be anticipated.

If erosion and other ravages of time are the cause of the missing record, one should expect the incompleteness to increase with age. This, however, is not the case and the explanation cannot be so simple. Saddler's voluminous data show that shortfalls do not only occur on all time scales but that they are a function of the time scale. Taking a hypothetical fluviatile sequence 500 m thick and spanning 7.5 Myr, he shows that a reasonable annual deposition rate, in the flood plain of a permanent river for example, will produce this thickness in 5,000 years. The observed sequence would thus be 0.075 per cent complete. If, on the other hand, we use a deposition rate on an hourly scale, a flash flood perhaps, the section's completeness would be 1 in 10 million.

The consequences of the inferred severe incompleteness of the geological record and its relation to time scale deserve our most serious attention because they affect the history of the Earth in many ways. Consider, for instance, the desire to locate the real boundary between two stratigraphical rock units. The likelihood that such a boundary will have been preserved is much greater for units of long duration than for brief ones: our chance of finding the boundary between two strata of 5 Myr duration in an interval of 25 Myr is much greater than that of locating the contact between two beds each spanning 1

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Myr. Conversely, if we believe that we have located the deposits associated with a brief event, an asteroid impact perhaps, in several stratigraphical sections, we ought to consider the extremely low probability that any event on a decadal time scale would be preserved at all, even once.

Many of the gaps in the record can be clearly seen, especially those on longer time scales, but the conclusion is inescapable that most breaks must go unnoticed. The geological record appears to consist of brief periods of activity separated by long times during which nothing happens or, alternatively, long times during which brief events leave minimal imprints which are removed by the next ephemeral event. However, even if only the occasional major event leaves a record substantial enough to be preserved, one would still like to think that the intervening times of erosion and non-deposition might leave some trace, a subtle alteration of the surface, a bit of soil formation, some burrowing. There is plenty of evidence that such effects exist and can be observed, yet most gaps must remain unmarked. I was much influenced early in my career by the recognition that two thin coal seams in Venezuela, separated by a foot of grey clay and deposited in a coastal swamp, were respectively of Lower Palaeocene and Upper Eocene age. The outcrops were excellent but even the closest inspection failed to turn up the precise position of that 15 Myr gap.

The geological record may thus be a record of rare events separated on any time scale by numerous and long gaps. Apart from the problems this presents in stratigraphy, there is no more cause for disquiet in this concept than there is in the atom model, also an object of little substance and much space. If the history of the Earth is mainly shaped by rare but major events, we need not greatly regret our inability to track the trivial day to day happenings. Intuitively, the notion seems plausible: nature all around us seems to change mostly by leaps. If at first sight such a record of history appears uncomfortably limited, like one of only battles and kings, two centuries of study have shown that it makes sense even in this abbreviated form.

There are, however, also disturbing aspects beyond the need of a vastly increased care in stratigraphy and chronology. Among the many ideas fermenting today in the study of evolution there is one, frequently heard, that ascribes the major evolutionary steps to a jump advance, a concentration of major change in a very brief interval of time. There seems to be no good reason why such pulses of evolutionary change should coincide with the major rare events that built the sedimentary sequence in which the record of evolution is contained. Thus, the new 'catastrophist' view of the sedimentary record implies that key elements of the evolutionary record may be forever out of reach. □

A potpourri of plucked patches

by Henry A. Lester

HAMILL, Marty, Neher, Sakmann and Sigworth[1] and Horn and Patlak[2] recently described new techniques for improved recording from single ion channels in biological membranes. They also showed how to excise the membrane patch under study, so that the experimenter can manipulate the solution bathing either the cytoplasmic or the external face. These methods, described in a *News and Views* article by McBurney[3], are leading to an explosion of new knowledge. The pace can be gauged by this review of six months' progress.

Studies on acetylcholine receptor channels have led the way. Two papers in this issue of *Nature* reveal that channel behaviour is more complicated than the former view of a simple transition to a unique open state with a smooth, featureless ion flux. First, Hamill and Sakmann (see p.462) shows that rat myotube channels have two open states that differ by several fold in their conductance. Channels open to the 'main' state, then hop (with a low probability) to the less conductive 'substate'. From the substate they can either close or return to the main level, with roughly equal probabilities. In addition to this wrinkle in the description of the open channels' *conductance*, the improved temporal resolution of the gigaseal technique has enabled studies on the fine structure of the *gating* events. The opening and closing transitions themselves require no more than 10μs so that complete events lasting just 50–70 μs can be resolved[1]. Colquhoun and Sakmann (see p.464) now report that the open state is indeed interrupted by events of this brevity when the agonist is suberyldicholine in frog muscle. On the average, a single open 'burst' is interrupted 3.1 times by gaps whose lifetimes are exponentially distributed with a mean of 150 μs. The investigators consider the possibility that these *Nachschläge* represent the long-sought transitions between the bi-liganded open and bi-liganded shut states. An additional, more subtle form of 'open-channel noise' can be observed in the patch-clamp records of other investigators. Such fluctuations probably do not arise at the single-ion level, but may be explained by motions of the amino acid side chains that presumably line the channel. If so, patch-clamp recordings provide an elegantly simple way to obtain information on the molecular dynamics of proteins.

The glutamate receptor channel of locust muscle weighs in with a hefty conductance of 125–150 pS. Recent patch-clamp investigations have sought to understand the basis for the sigmoid dose–response curve. With an elegant multibarrel internal perfusion system for the patch electrode, Cull-Candy *et al.*[4] found that the channel opening rate increases with the square of the glutamate concentration, suggesting that two glutamate molecules are required to open a single channel. Gration *et al.*[5] found that the mean channel duration also increases with [glutamate] as though all sites must be vacated to allow channel closing. It is not clear whether these two factors are compatible with each other; it is perhaps relevant that Gration *et al.* observed non-random features in the gating kinetics, suggesting as yet unexplained transitions in agonist affinity.

Electrically excitable Na^+ channels are rather more difficult to study carefully because the open conductance is only 6–18 pS; depending on the conditions. Nonetheless it seems clear from the available recordings that the open Na^+ channel does not flicker[6,7]. Horne *et al.*[7]. compared the closing of channels which had opened either early or late during a depolarizing clamp pulse and obtained evidence for the view that activation and inactivation are independent processes, as in the original Hodgkin–Huxley formulation. However, macroscopic voltage-clamp experiments have for some time pointed to discrepancies between observed kinetic behaviour and that predicted by the Hodgkin–Huxley equations; and patch-clamp investigations now confirm that the open state lasts longer than would be predicted if the channel could be closed with equal probability by transitions in any of three putative subunits. Apparently the closing rate is limited by the last transition preceding the open state in the activation pathway, a conclusion that is particularly clear when the inactivation pathway has been removed by exposure to *N*-bromoacetamide[8].

In almost every type of cell that has been patch-clamped, the investigators were startled by enormous signals that took the oscilloscope trace off screen. Upon further investigation these signals were recognized as Ca^{2+}-activated K^+ channels (chromaffin cells[9], myotubes[10], autonomic ganglia[11], *Helix* neurones[19] and human erythrocytes[12]). These channels have conductances of 100–200 pS and show very interesting flicker and open channel noise. The opening rate increases with both $[Ca^{2+}]$ and depolarization, with an absolute requirement for Ca^{2+}, as though the membrane potential is influencing the effective affinity constant for Ca^{2+}. It would be most interesting to know (a) the Hill coefficient for the dose–response curve and (b) whether the channel still functions after procedures that remove calmodulin from the membrane.

Other potassium channels have been observed with the patch clamp. Ohmori *et*

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al.[13] recorded inward ('anomalous') rectifier channels on myotubes as they flickered due to the binding and dissociation of Ba^{2+} ions. The conductance (10pS), kinetics and voltage sensitivities agree well with results from noise and voltage-jump relaxations. However, at the recent Neuroscience meetings H.C. Hartell reported that muscarinic agonists induce surprisingly brief K^+ channels in cultured heart muscle. The average duration is 37ms or about a tenth as long as the time constant derived from fluctuations[14], voltage-jump relaxations[15], or concentration-jump relaxations[16]. The channel conductance is 8pS. Hartzell speculates that the slow macroscopic relaxations derive from repeated binding of agonist to receptors, leading to multiple channel activations.

There's more. Bormann *et al.*[17] recorded single GABA-sensitive channels in excised membrane patches from cultured spinal cord neurones. The channels are selective for chloride and have a conductance of 23pS. And Lux and Nagy[18] observed single Ca^{2+} channels in *Helix* neurones. The opening rate increases with depolarization; the channel duration (about 25ms), on the other hand, shows no voltage dependence. The conductance of 5–15 pS is much larger than expected from noise measurements but exact comparisons could be misleading because the Ca^{2+} channel is not ohmic.

Patch-clamp recording are so easy (compared with conventional voltage clamp experiments), and so immediately gratifying, that we are incorporating them into an undergraduate laboratory course at Caltech. The only specialized instruments required are a high-quality microforge and a carefully engineered headstage (now available from several manufactures). Data analysis is tedious and time-consuming, but this will undoubtedly not prevent a host of interesting and important investigations with the new techniques. □

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T lymphocytes: repertoire and recognition

from Nigel Williams

ANY discussion of T cell immunology sooner or later leads to controversy. Although T cells, like B cells, are capable of recognizing foreign antigens with exquisite specificity, the nature of their activation is far less well understood. First, they are a heterogeneous population pursuing both effector and regulatory functions, recognizing mainly membrane-bound antigens, and second, they are restricted to seeing antigens in the context of molecules of the major histocompatability complex (MHC). This has been one of the most exciting findings in immunology in recent years and raises questions of how both a foreign antigen and a MHC molecule in the same membrane can be recognized by a single T cell and why activation results only when both are present. Whether one or two receptors are required for the recognition step is hotly debated and the mechanism by which such a sophisticated repertoire develops within each individual is also in dispute. It was with these two major questions in mind that many workers in the field were recently invited to a workshop in Rüdesheim in Germany*.

Ontogeny of the T cell repertoire

The T cell repertoire is constrained both by the requirement of MHC restriction and by the need to remove or inactivate, early in development, T cells capable of responding to normal antigens found on the cells of the individual. This 'learning' process has been believed to occur in the thymus but recent studies of the congenitally athymic nude mouse are raising some questions. Nude mice have T precursor cells but the development of the repertoire is deficient. H. Wagner (Johannes-Gütenberg University, Mainz) and T. Hünig (University Würtzburg) reported the generation *in vitro* of cytotoxic T cells from precursors found in both normal and nude mice, but the nude mouse was deficient in precursors against certain antigens. J.C. Cerottini (Luding Institute), using similar techniques, found only quantitative differences between the two animals although nobody has yet been able conclusively to demonstrate functional helper T cells in the nude mouse. The precise immunological status of the nude mouse is therefore still not clear but the existence of at least some precursor T cells which can be stimulated *in vitro* suggests that some pre-thymic development of the repertoire may occur. A. Singer (NIH) described experiments specifically designed to test this hypothesis. He constructed chimaeras using an $(A \times B)$ F_1 host, thymectomized, lethally irradiated and grafted with an A thymus and A bone marrow, whose recovered thymocytes were shown to be tolerant to B MHC determinants. This suggested that tolerance had been induced prethymically and some pre-T cells at least must already have learned self MHC. Support for extra-thymic development of some precursor T cells also came from experiments reported by R. Miller (Ontario Cancer Institute, Toronto) who described a novel culture system for generating T cell colonies from single immature precursor T cells which were MHC restricted but surprisingly developed several antigen specifities.

On the other hand, transplantation experiments have shown that the thymus does exert a significant but as yet unclear effect on the generation of the T cell repertoire. It is thought that the thymic epithelium may act by presenting antigens and self-MHC molecules to T precursor cells after which selected clones are stimulated to develop to maturity. I. Weissman (Stanford University) presented provocative data on the discrete distribution of different classes of MHC molecules in different regions of the thymus, but there is as yet no firm evidence of a common pathway by which T cells pass through the thymus and undergo step by step acquisition of the repertoire in the normal animal. Clearly new approaches are required and R. Jordan (Newcastle University) presented interesting preliminary experiments using cold treatment of thymuses *in vitro* to obtain pure thymic epithelium which may be useful in understanding its role in antigen presentation.

The picture emerging seems to be that the thymus acts on T cells to select precursors which are restricted to recognizing antigen in conjunction with self-MHC molecules and to expand this population, possibly by an interleukin-2 signal. Some clones, however, may develop outside the thymus without having been selected to see antigen only in conjunction with self-MHC molecules. It may be that this population does not receive an inductive signal and so remains in low numbers in the normal animal, overshadowed by the thymic-processed, self MHC-restricted population.

The experiments described by Z. Nagy (Max-Planck-Institut, Tubingen) were very interesting in this respect. He has been looking at the phenomenon of low responsiveness which is found in specific mouse strains against certain antigens. In an *in vitro* system he has shown that T cells from a low responder strain can respond to the antigen if it is presented by (non-self) antigen-presenting cells. This suggests that there are two compartments in the T cell repertoire, one that recognizes foreign

*A workshop on 'The role of the thymus in the T cell repertoire' was held in Ruderheim on 16–19 September 1981 organised by the Johannes-Gütenberg-Universitat, Mainz and sponsored by the Deutsche Forschungsgemeinschaft.

Nigel Williams is a member of the editorial staff of Nature.

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antigens in the context of self-MHC molecules and another that can use allogeneic MHC molecules of the species for the context of antigen recognition. However, it seems that in the mice of the low responder strain, clones capable of responding to the antigen in the context of self MHC are absent. Such experiments emphasize the importance of MHC molecules in the generation of the repertoire and raise difficult questions about the nature of antigen recognition by T cells.

T cell–antigen interaction

There are two opposing theories of how individual T cells recognize both MHC molecules and a foreign antigen (X) — one model requires two separate receptors and the other, the interaction antigen model, has recently been argued by Matzinger (*Nature* **292**; 497, 1981). This model proposes that MHC molecules interact with antigen X and are presented as a single entity to a single T cell receptor. In its most provocative form it proposes that the interaction of specific self-MHC molecules with a particular antigen X will give rise to a new antigenic determinant expressed only by the interacting complex. A prediction of the interaction antigen model is that cross-reactivity with allogeneic MHC + antigen Y could occur, for example if the allogeneic MHC + antigen Y interaction produced an antigenic determinant cross-reactive with those of a self MHC + antigen X interaction. Since the cross-reactive determinants are absent from the individual non-interacting components, then if the T cell recognizes both entities via separate receptors cross-reactivity would not be expected. T. Hünig (see this week's *Nature*, p.460) presented experiments showing that indeed a small proportion of clones of T cells specific for self MHC + X showed reactivity against allogeneic MHC + Y under some conditions. R. Schwartz (NIH) and C. Janeway (Yale University) also reported the generation of self MHC-restricted clones specific for antigen X which showed a degree of cross-reactivity with allogeneic MHC molecules.

Another prediction of the interaction antigen model is that T cells will have no particular specificity for self-MHC molecules or antigen X alone, being specific only for a combination of the two components, whereas with the two receptor model T cells should bear separate receptors for antigen X and for MHC molecules. H. Cantor (Harvard University) presented data from a T cell clone specific for self MHC + X (in this case, cow insulin) which secreted a 68K molecule into the supernatant. The molecule bound to other related insulins with a lower but particular affinity — when passed through columns each containing a different insulin, it was recovered in the same proportion as predicted by its binding affinities. A column containing the specific restricting MHC molecules failed to bind the molecule. If this molecule is part of the T cell receptor (which is uncertain at this stage), then a degree of independence of the antigen-binding component and the component specific for the restricting MHC molecules is suggested. If the two components are indeed presented as an interaction antigen it must be of the very loosest kind. R. Schwartz interpreted these data in terms of a single receptor which has two separate binding sites with different specificities. This could possibly explain how a receptor specific for self MHC + X could recognize allogeneic MHC molecules if these were sufficiently structurally different to make contact with both binding sites sufficient to activate the cell.

Clearly we need to know a great deal more about the precise role of MHC molecules in T cell responsiveness. To this end G. Hämmerling (Cancer Research Center, Heidelberg) showed that different T killer cell clones restricted to the same MHC molecule could be blocked from responding by monoclonal antibodies to different determinants on the MHC molecule. Until we know more precisely the function of MHC molecules, the manner in which a single T cell sees both MHC molecules and a foreign antigen is likely to remain controversial and immunologists will still not know if 1 + 1 = 1 or 2. □

Receptors, channels and whole-body NMR in Mexico

from M.J. Geisow and J. Mas-Oliva

THE 7th International Biophysics Congress and 3rd Pan-American Biochemistry Congress was held in Mexico City recently. Despite the title, no dichotomy was obvious — it is now impossible to distinguish biophysicists from biochemists on the basis of project or hardware. Many topics were covered, but a dominant theme was the measurement, biochemical analysis and functional implications of ionic gradients across cell membranes.

An understanding of transduction or ion transport through biomembranes can only come through knowledge of the structure of intrinsic membrane proteins. As yet, no example exists, whether receptor, channel or carrier, where structural determination is sufficiently advanced to allow firm conclusions about mechanism. This situation was not changed in Mexico, although bacteriorhodopsin is clearly a front runner with a tentative assignment of amino acids to the 3Å analysis and extensive work on reconstituted rhodopsin systems.

Also promising is the F_1 structure of proton-translocating ATPase ($F_1.F_0$) reported by Amzel *et al.* (Johns Hopkins University) at 9Å resolution. Three large domains, related to a similar triplet by local symmetry, seem likely to reveal the location of the five types of polypeptide as well as the ligand and effector sites. The protonophoric (F_0) membrane-embedded chains may consist of a hairpin arrangement of twin helices (Sebald and Hoppe, Technical University of Braunschweig). They bear a buried, invariant acidic amino acid the loss of which, by modification or mutation, halts proton translocation.

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Another close contender is the acetylcholine (ACh) receptor, reviewed by J.P. Changeux (Pasteur Institute, Paris). The minimum functional aggregate (in reconstitution experiments) consists of four glycosylated peptides: α(40K), β(50K), γ(60K) and δ (65K) in 2:1:1:1 stoichiometry and having NH_2-terminal sequence homology. α is labelled by ACh-reactive analogues, β by channel blockers (such as histrionicotoxin). Three molecular transitions of the complex, revealed by bound fluorescent agonists, may be associated with a minimum of three pharmacological 'states' of the receptor.

The sodium channel of neuronal plasma membranes is more elusive. Miller and Agnew (University of Colorado) have studied a 260,000-M_r tetrodotoxin-binding component of the channel complex. The binding properties of this highly charged glycoprotein are particularly sensitive to the lipid microenvironment.

Working with sarcolemma, Almers *et al.* (University of Washington) established that sodium channels are relatively immobile in the bilayer. Their local destruction in a clamped patch of membrane by UV photobleaching leads to loss of sodium conductance for one hour. Channels are refractory to laterally applied electric fields and are considered to be 3 orders of magnitude less mobile than rhodopsin.

The strong probability that other channels and receptors are anchored to the cytoskeleton also emerged. The ACh receptor seems to be fixed so firmly in the membrane by an alkali-extractable 43,000-M_r component (not actin) that the addition of exogenous lipid leads to rafts of receptor aggregates, rather than randomly dispersed molecules. Cherksey and Zadunaisky (State University of New York) used the fluorescence of propanalol

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as a probe for the mobility of the β-adrenergic receptor in the presence and absence of microfilament-disrupting drugs and conclude that the receptor is anchored to the cytoskeleton.

A steady improvement in the quality of 'model' membranes is taking place at the same time as the successful extraction and characterization of intrinsic membrane proteins. M. Montal (University of California) has developed a technique of spreading two monolayers of protein and lipid and apposing them across an aperture to form a planar bilayer. H. Schindler (University of Basel) employs the same technology, but has discovered that natural membrane or lipid vesicles, added to the aqueous compartments, are in equilibrium with and contribute representative lipids and proteins to the surface monolayers.

The functional reconstitution of membrane proteins will allow two persistant problems to be addressed. First, what, if any, is the influence of particular lipids on protein function? and second, what role is played by protein–protein interaction in the bilayer? P.F. Devaux (Pasteur Institute, Paris) has devised spin-labelled lipid probes which covalently attach via their head groups to reconstituted membrane proteins. This achieves a significant increase in occupancy by probe of the protein–lipid boundary. Rotational correlation times of the proteins obtained in this way indicate strong lateral interactions (protein–protein) for ACh receptor and Ca^{2+}-ATPase and very weak ones for rhodopsin. Spin exchange between boundary probe and lipids spin-labelled with a different nitrogen isotope show that migration of lipids into the lipid boundary of rhodopsin is independent of the head group.

Lateral interactions between the intrinsic membrane proteins of the inner mitochondrial membrane were explored by C.R. Hackenbrock (University of North Carolina). Electron transfer between the various oxidation–reduction complexes and carriers appears to be facilitated by the gregarious nature of the proteins. However, although electron transfer decreases monotonically with the addition, by fusion, of exogenous lipid, it does not cease. Freeze-fracture of vesicles reveals uniform dispersion of all particles (proteins) in lipid-enriched membrane vesicles. Antibody marker techniques demonstrate that cytochromes oxidase, *b* and *c* do not migrate as complexes. In fact, sufficient diffusional motion (measured by freeze-fracture of vesicles after relaxation of an electric field) of the intrinsic proteins can occur within one turnover of reducing equivalents to render stable protein–protein interactions unnecessary.

Another role hypothetically associated with lateral interactions of intrinsic membrane proteins is the formation of an ion pore/channel in response to an associating stimulus. Montal and Borochov-Neori (University of California) examined whether vertebrate rhodopsin mediates changes in membrane ion permeability by aggregation following the light-dependent conformational change of the molecule. Conductance characteristics of planar bilayers containing rhodopsin support this view of channel formation. However, the alteration in aggregation state after bleaching (determined by fluorescence-energy transfer between rhodopsin monomers) is more subtle than expected.

Cast in the role of principal information carrier associated with the operation of membrane channels, ionic calcium continues to attract much attention. The eager studies of the action of intracellular calcium have often overshadowed the more sober, but hardly less important, investigation of its intracellular regulation. Although there is still confusion and controversy about how calcium enters the cytoplasm, steady progress has taken place in measuring it when it is there and on how it is removed to return the cell to a resting state. For example, using permeant fluorescent calcium indicators synthesized by R.Y. Tsien (University of Cambridge), resting lymphocyte calcium ion was measured at 0.12 μM.

For the extrusion of calcium ions, two plasma-membrane processes are available: a Na^+/Ca^{2+} exchange protein and a calcium-stimulated ATPase. Blaustein and Nelson (University of Maryland), DiPoldo and Beauge (Caracas and Cordoba, Argentina) and Caroni and Carafoli (University of Zurich) all described a calcium removal process operating at very low levels of Ca^{2+} (down to 0.1μM), fueled by ATP. However at high Ca^{2+} (up to 10 μM), Na^+/Ca^{2+} exchange was dominant. The latter authors reported that the cardiac muscle sarcolemma Na^+/Ca^{2+} exchanger swops $3Na^+$ for 1 Ca^{2+} (requiring a counterion) and is insensitive to calmodulin, unlike the Ca^{2+}-ATPase.

High intracellular Ca^{2+} was formerly thought to regulate the permeability of the intercellular gap junction. As related by M.V.L. Bennett (State University of New York), the association constant for this process is unphysiologically high (K = 0.4mM Ca^{2+}). In single pairs of embryo blastomeres connected by gap junctions, conductance is regulated by a process with a pK_a of 7.3 (the channels are closed when the cytoplasm is slightly acidic). Voltage clamping the cells at different levels, thus forcing a transjunctional potential, also closes the channels. These two control processes are not the same, since aldehydes selectively block the pH dependence.

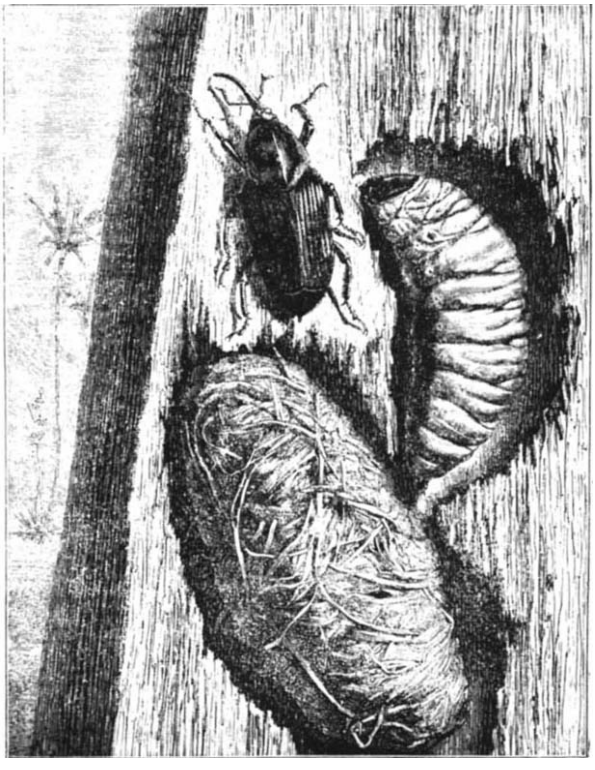
Work on the mammalian liver cell gap junction has also progressed well; a hexameric array of protein units spans the

100 years ago

NATURE

POPULAR NATURAL HISTORY

We give, through the courtesy of the publishers, another illustration taken from the chapter on Weevils. It is of a weevil known as *Rhyncophorus palmarum*. Its fat grubs live on the stems of palm-trees, and are often very destructive. Several of the species are very injurious to the sugar-cane. One found in sugar-plantations in Guiana contain in their intestines lumps of a sweet waxy substance — the altered saccharine food on which they live — and for this they are boiled and eaten by the native. The fine fat larva and the pupal condition, as well as the full-grown weevil, are to be seen in the engraving.



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membranes of the communicating cells. B. Zampighi (UCLA) described a new junction for eye lens fibre membranes. At low resolution, the protein subunits are tetramerically arranged, unlike the conventional gap junction.

Many functions of intrinsic membrane proteins depend on ATP, and in the last session delegates were rewarded with the sight of NMR spectrometers built to measure ATP in most parts of Britton Chance's anatomy. The impact of this lecture was certainly magnetic and the closing slides of the ultimate biophysical conjunction, man and machine, were a fitting way to end the Congress. □

Control of a mixed tRNA–protein operon

from Andrew Travers

DURING exponential growth the bacterium *Escherichia coli* balances the production of ribosomes and other necessary components of the translation machinery of the cell so that no more ribosomes are produced than are required for the maintenance of growth. To achieve this balancing act the synthesis of the stable RNA species, rRNA and tRNA, must be accurately coordinated with the synthesis of both ribosomal proteins and the cycling translation factors. One such protein is the elongation factor Tu (EF-Tu) whose function is to bind charged tRNA and facilitate its engagement in the acceptor site on the ribosome as a prelude to peptide bond formation. Accordingly, the amount of EF-Tu in the cell parallels that of tRNA. The protein is one of the most abundant in *E. coli* comprising up to five per cent of the total cellular protein and is encoded by two distinct genes, *tufA* and *tuf*B, whose products differ by only a single amino acid[1,2].

The major gene for EF-Tu, *tufA*, is expressed at ~ 2.5 times the rate of *tufB*, and is located in the centre of a cluster of r-protein genes with which it is co-transcribed. This arrangement is typical of r-protein genes in general. By contrast, recent work by three groups[3-5] has demonstrated that *tufB* is not associated with other r-protein genes but instead is co-transcribed with a neighbouring group of four tRNA genes, *thrU, tyrU, glyT* and *thrT*. This transcription is directed by a promoter lying upstream of the tRNA genes and is terminated immediately distal to the *tufB* gene. Although co-transcription of tRNA and protein genes has previously been observed in mitochondria this is the first report of this type of gene organisation on the bacterial chromosome. Moreover Hudson *et al.* (see this issue of *Nature* p.422) suggest that this example is not unique and that the *tyrT* transcript may encode both two $tRNA_1^{Tyr}$ sequences and a small arginine rich protein. In this case, however, the evidence for the production of such a protein *in vitro* remains to be established.

What is the functional significance of the co-transcription of stable RNA and r-protein genes? Hudson *et al.* advance two possibilities. The presence of a tRNA structure immediately preceding the *tufB* coding sequence could enhance the stability of the *tufB* mRNA. For this hypothesis the *in vivo* evidence offers as yet little support. Alternatively, the co-transcription of tRNA and mRNA would result in a measure of coordinate regulation. The transcription of both stable RNA and r-protein mRNA species is regulated both by the availability of amino acids and by variation in growth rate. *In vivo*, sudden amino acid depletion results in a drastic curtailment of the production of all major components of the translation machinery. This stringent control is mediated largely at the level of RNA chain initiation and is associated with the presence of a G+C-rich discriminator sequence located close to the start point of transcription.

In addition, promoters regulated in this mode often, but not invariably, contain extensive regions of dyad symmetry. The promoter for the tRNA — *tufB* transcription unit contains both these features and is indeed controlled stringently both *in vivo*[6] and *in vitro*[4].

For control by amino acid availability, the co-transcription of stable RNA and an r-protein mRNA is logical. However, a paradox is apparent when the linkage of transcript production to growth rate is considered. As the growth rate of a bacterial culture increases there is a proportionate increase in the number of ribosomes per unit amount of total cellular protein. Since the ratio of production of total protein increases in direct proportion to the growth rate (μ) the synthesis rate of both stable RNA and r-protein must increase approximately in proportion to μ^2 to yield a preferential enrichment of ribosomes. However, whereas the transcription of both rRNA and the most abundant tRNA species follows this latter pattern that of r-protein mRNA does not, increasing only in direct proportion to μ (ref.7). Thus the growth rate dependent regulation of r-protein synthesis is probably mediated by post-transcriptional controls, including the feedback inhibition of r-protein mRNA translation by free r-proteins. By fusing rRNA and r-protein promoters to the coding sequence for galactokinase or B-galactosidase Miura *et al.*[8] have recently demonstrated that the growth rate dependent regulation of transcription is programmed by the promoter region and thus probably reflects variation in rate of RNA chain initiation.

This pattern of differential initiation at stable RNA and r-protein promoters raises the question of whether the growth rate regulation of transcription of the mixed tRNA-*tufB* operon resembles that of r-protein mRNA or of stable RNA. Whatever the answer it is apparent that regulation of at least one portion of the operon would be anomalous.

No direct evidence bears on the question nor does the *tuf*B promoter sequence offer any obvious clues. However, the function of the tRNA products of the operons favours the possibility that the whole operon is regulated in the r-protein mode. At high growth rates the r-proteins comprise the major class of proteins synthesised in the bacterial cell. Their translation, as well as that of EF-Tu itself,[1,2] preferentially utilises a limited subset of possible codons. Thus those tRNA species which recognise the codons included in this subset will be required in high amounts at high growth rates. Of the tRNA species contained within the hybrid operon two, the products of *thr*U and *gly*T, do not utilize the preferred codons. A third, the product of *thr*T, does recognise codons in this class but is the minor species of the two $tRNA^{Thr}$ isoacceptors which do so. Thus there is no obvious requirement for the products of these three tRNA genes to be preferentially synthesised at high growth rates. However, the product of the fourth tRNA gene, *tyrU* also utilizes a preferred codon and is 1.5 times as abundant as the product of the second $tRNA^{Tyr}$ gene, *tyr*T. Nevertheless, the *tyrT* gene contains two copies of the tRNA sequence in a single transcription unit, an arrangement similar to that for the major $tRNA^{Gly}$, a tRNA utilized preferentially at high growth rates. Thus with the possible exception of the *tyrU* product all the products of the mixed operon, both *tRNA* and protein are minor components of mixtures of molecules of similar function. Such a situation would be more compatible with the pattern of r-protein regulation than with that of stable RNA regulation. In accord with this possibility the production of the *tufB* is less sensitive to amino acid depletion than is the *tufA* product[6] and so would be favoured under conditions of growth rate limitation imposed by lack of amino acids. □

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The geological exploration of Tibet

from A.M.Celâl Sengör

OWING to its unique physiography, geographical location and culture, the Tibetan Plateau has long attracted international scientific interest. Tibet owes its uniqueness to its very high elevation (~ 5 km above sea level), its vast areal extent (~ 2.5 million km^2) and its position in the rain shadow of the Himalayan Range (Fig.1). Tibet's elevation has been attributed to a double thickness (~ 70 km) of continental crust underlying it[1], but the precise mechanism for the development of this thick crust and the elevated plateau has been hotly debated. The lack of data from Tibet has been the chief factor in allowing widely divergent opinions to be held on even the most critical scientific questions concerning it.

A number of European and Sino-European scientific expeditions penetrated Tibet during the last decade of the 19th and the first four decades of the 20th century and gathered a large amount of data, pertaining to various disciplines, before such expeditions became impossible owing to the political changes in the area. The great scientific value of Tibet is appreciated by the Peoples' Republic of China, as evidenced by the number of diverse Chinese expeditions devoted to the exploration of Tibet since 1951. From 25th May to 14 June 14th, 1980, Academia Sinica organized a multidisciplinary, international symposium, including a field excursion on the Tibetan Plateau, both to report the results of the three-decade-long Chinese studies to the international scientific forum and to exchange opinions with their foreign colleagues[2,3]. The symposium ended with mutual wishes for future scientific collaboration.

The three papers in this issue of *Nature* (see p.405–417) by a Franco-Chinese team report the preliminary results of a three-month field trip in Tibet in the summer of 1980 and represent the first fruits of the renewed cooperative efforts of Chinese and foreign scientists in Tibet. Because so few foreign scientists have been to Tibet, let alone worked in it, the Franco-Chinese co-operation and the publication of its initial results in a language easily accessible to foreigners is of great importance.

The problems that most students of Tibetan geology are currently trying to address can be divided into two major groups. (1) Although there is general agreement that the basement of the Tibetan Plateau is composed of a small number (two?) of blocks that detached from the northern periphery of Gondwanaland and subsequently accreted to Asia, the exact history of these events is still a matter of conjecture. The elucidation of this history and the sequence of events that accompanied the India–Eurasia convergence, and their precise timing, constitute what I call the first-order *palaeotectonic* problems of Tibetan geology. (2) It is clear that the evolution of the present Tibetan high plateau is somehow related to India Eurasia collision during the early Tertiary[1], but exactly how has been and still remains the question. This seems to be the first-order *neotectonic* problem of Tibetan geology. The three papers in this issue deal with both groups of problems in their study areas and also make some generalizations that pertain to the entire plateau.

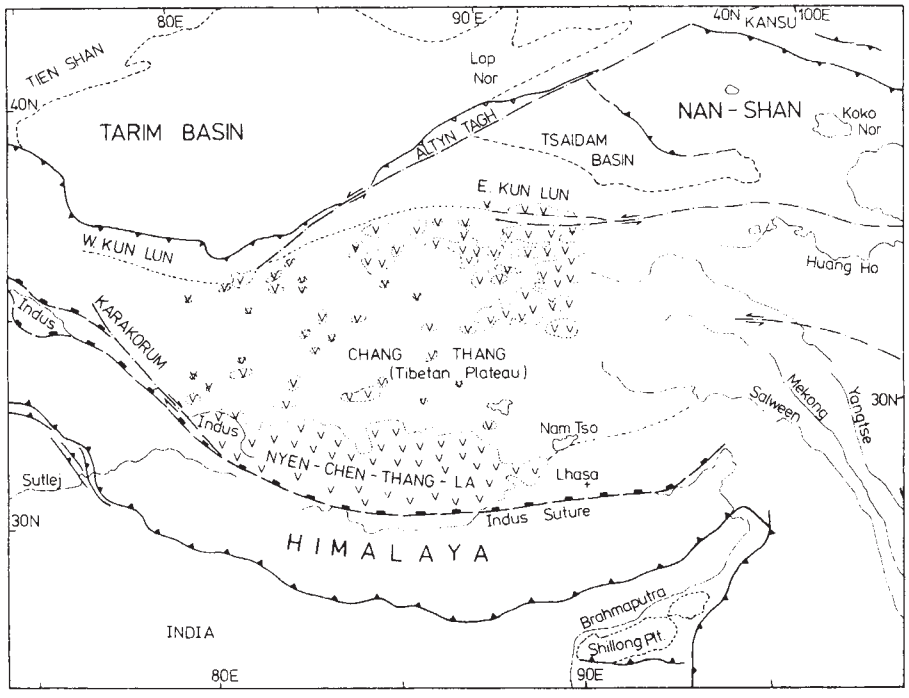

Fig. 1 Tectonic sketch map of the Tibetan Plateau and surrounding areas. Lines with black teeth are active thrusts around the plateau, lines with open teeth are inactive thrust boundary. v = Neogene and younger volcanic rocks (from ref. 11).

Tapponnier *et al.* (p.405) review the background and general results of the cooperative field-work in southeastern Tibet. Their stratigraphical observations on the Lhasa Block are consistent with the earlier inference that this block, bounded in the north by the Tanggula suture of late Jurassic–early Cretaceous age and in the south by the Yarlung-Zangbo suture of early Tertiary age, was, until the Triassic, a part of Gondwanaland. The most interesting novelties reported are those that pertain to the evolution of the Yarlung-Zangbo suture, the lineament that marks the line of apposition between the Indian sub-continent and Eurasia following the late Cretaceous–early Tertiary demise of Neo-Tethys. Tapponnier *et al.* argue that the structural evolution of the Yarlung-Zangbo suture involved four distinct episodes of deformation in the region they studied. Although the precise timing of these episodes and that of the emplacement of the Yarlung-Zangbo ophiolites remains controversial, the kinematic sequence they describe is a considerable advance. Their discovery of the apparent continuity between the epi-ophiolitic pelagic sediments and the unconformable clastic cover of the Gangdise granodiorites strongly supports the earlier interpretations of the Xigatse basin as a fore-arc basin[2,4] and indicates that it probably had an ophiolitic basement. They also underline the importance of the distinction between the Triassic passive continental rise aprons[2] (under the unfortunate designation "Triassic Flysch") and the Cretaceous wildflysch linked to orogenic deformation. Although there is sufficient evidence to the contrary, Tapponnier *et al.* reiterate the peculiar view that the Gangdise Andean-type arc might equally well have been an island arc!

A.M. Celâl Sengör is in the Geology Department of the Technical University of Istanbul and would like to thank Esen Arpat for his help in the preparation of this account.

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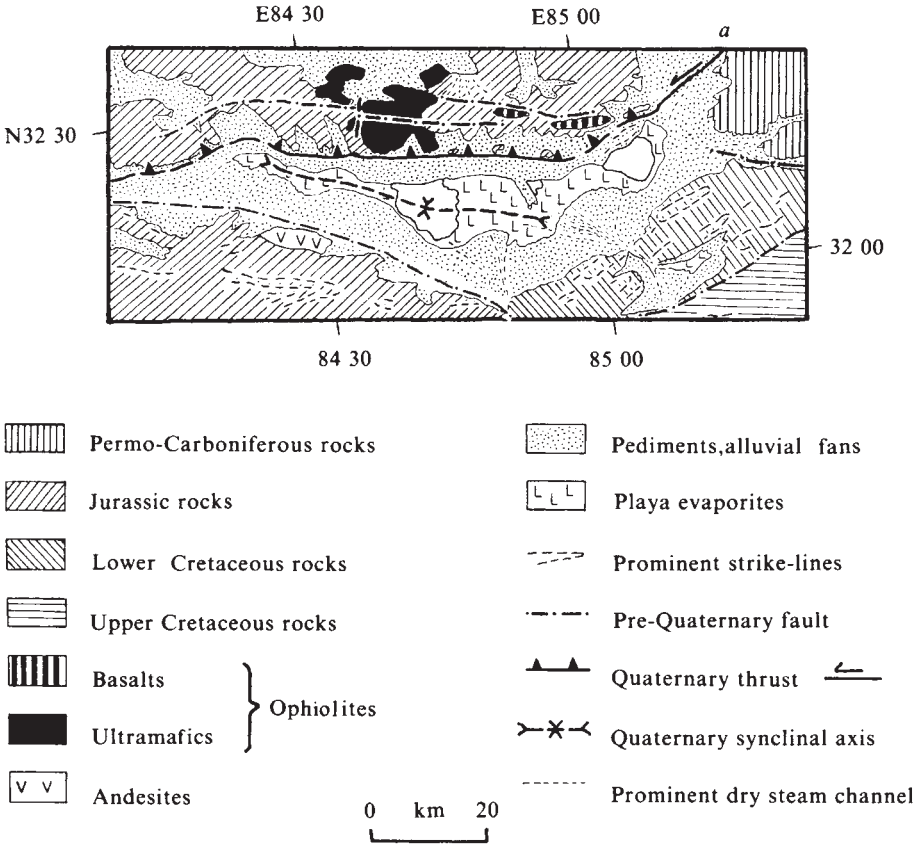

Fig.2 1:1,000,000 geological map of the Zhaxi Co area drawn from Landsat image no. E-2664-04045-6 01 and sheet 6 of ref.15. White areas are present lakes. *a* is the strike-slip fault that seemingly connects with the Zhaxi Co thrust, the north-east continuation of which is particularly visible on Landsat image no. E-2663-03584-6 02.

The ophiolites of the suture zone themselves, as exemplified by the Xigatse ophiolite massifs, are interpreted to be unusual examples in the paper by Nicolas *et al.* (see p.414 of this issue). They note the greatly reduced thickness of the mafic part of the sequence, which is almost devoid of cumulate gabbros. The serpentinized ultramafic part is invaded by numerous diabase sills after the onset of serpentinization. Nicolas *et al.* prefer to interpret the Xigatse ophiolite as a possible product of a very slowly spreading ridge, although some of the reported peculiarities of this ophiolite complex, particularly the thin mafic section and the late intrusion of diabase into the ultramafic foundation invite suspicion in terms of a near-fracture zone ophiolite[5,6].

Finally, both papers by Tapponnier *et al.* emphasize the east–west extensional nature of the Quaternary and also possibly late Tertiary deformation both in the region visited in particular and in Tibet in general, in full agreement with the long declared view of some of the French members of the team[7,8]. They note the decreasing intensity of north-south compressional deformation in southern Tibet since the late Cretaceous and underline the total absence of any Quaternary compressional features such as thrusts or folds in the areas studied. The significance of these preliminary observations increases by the fact that they seem to contradict the views of some Chinese[9] and foreign[10,11] workers who argue for the existence of Quaternary north–south shortening in Tibet. In fact the most recent geological map of Tibet[12] shows a number of east–west striking faults cutting Quaternary deposits in southern Tibet and a few folded areas of Quaternary limnic and fluvial sediments. Figure 2 shows perhaps the most convincing case for Quaternary (active ?) thrusting in Tibet, in the area of Zhaxi Co. On sheet 6 of the 1:1,5 million geological map of Tibet[12], Zhaxi Co is shown to have perched in a Quaternary syncline. On Landsat images the Zhaxi Co depression is seen to be overriden from the north along a north-concave thrust, which, towards the east, seems to connect with a very prominent east-north-east-striking, left-lateral strike-slip fault (*a* in Fig. 2) with several sag ponds, dry stream channels bent in an anti-clockwise fashion and a very pronounced rift topography. All these structures are very young, if not active. The Zhaxi Co thrust may possibly continue westwards, in the direction of Gerze. Towards the east it is on strike with Siling Co, which is a Neogene (and younger ?) ramp basin (Chen Zhi-ming, personal communication, 1980), and with the 1972. 7. 22. earthquake (epicentre 31.4 °N, 91.5 °E) which occurred on an east–west thrust[13]. All of these very young thrusts seem to be aligned along the trace of the Tanggula suture and may represent its local, young reactivation. All such observations clearly indicate that the statement "in the Quaternary, deformation of Tibet was characterized by east–west extension" (Tapponnier *et al.* p.405) may not be descriptive of the entire picture and the comparison of Tibet with such dominantly extensional regions as the Basin and Range and the Aegean, both characterized by thinned crust, may not be exactly appropriate.

Tapponnier *et al.* also emphasize the "comparatively small amount of shortening and deformation of the surface sediments" north of the Yarlung-Zangbo suture. It would be of great interest to see the actual data and their quantitative results regarding local, observed values of stratal shortening. Because compressive deformation that results in buckle folding is often accompanied by about 10 per cent bulk shortening and thickening before folding occurs, such observations on folding alone always render a minimum value for shortening.

In conclusion, one cannot over-emphasize the significance of the recent resumption of Chinese and foreign co-operation in the geological exploration of Tibet. As the papers in this issue and this commentary clearly show, Tibet has a multitude of unsolved yet critical geological problems, the better understanding of which would not only further our knowledge of the regional geology and evolution of this unique structure but would also contribute greatly to our understanding of such fundamental geological problems as continental collision, behaviour of the continental lithosphere, differentiation of the continental crust, and a host of other, smaller ones. No amount of experience and expertise could be too much to bear on such gigantic and diverse problems and if only on these grounds alone the work of the Franco-Chinese team is to be applauded. One hopes it will continue, and other similar efforts be made in the future, to illuminate both the palaeo-and neotectonic problems of Tibet. □

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ARTICLES

The Tibetan side of the India–Eurasia collision

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Results are reported from the 1980 joint French–Chinese field expedition in Tibet. The area covered was from the High Himalaya in the south, to the region of Nagqu ~250 km north of Yangbajain. Ophiolites in the Zangbo valley represent remnants of the crust of an ocean basin which lay adjacent to the Gangdise granodiorite belt in the late Mesozoic. The ophiolites were thrust to the south onto the Cretaceous mélange and Triassic flysch but the age of this event is unclear. In the Tertiary, sediments of the Indian margin and the Xigaze basin were folded with steeply dipping cleavage, which indicates some north–south shortening of the crust. However, the Tertiary volcanic sequence on the Lhasa block and further north shows comparatively little folding and thrusting. In the Quaternary, deformation of Tibet was characterized by east–west extension.

TIBET has been much studied by geoscientists of the People's Republic of China, particularly in the past decade. Important results of these studies have been presented in two recent symposia. These results[1–7] and new maps at a scale of 1/1,500,000 (produced by the Ministry of Geology) provide a good geological data base for a country which is otherwise largely unknown in the west.

The French–Chinese joint study of the 'geological structure, formation and evolution of the Earth crust and upper mantle of the Himalayas' which began with three months of field work in the summer of 1980 provided the first opportunity for western scientists to work in Tibet since explorations at the beginning of this century[8–12]. Hence the results presented here are merely an extension of the Chinese work (see, for example, refs 13–15).

We summarize here preliminary field reports jointly prepared by French and Chinese geoscientists. More detailed accounts will be published elsewhere[16–20]. The joint study was limited to a region north of the Himalayas between Zham and Lhozhag, and south-west of Nagqu (~250 km north of Yangbajain). [Note that geographical names are spelled according to the Ping Yin transcription on the most recent maps published in the People's Republic of China: Jiang means river in Chinese and Co, lake in Tibetan.] Some of us (P.T., F.P., X.X.C.) went close to the border with Qinghai, on a reconnaissance trip.

Several important questions about the evolution of Tibet remain essentially unanswered. For example, although there is little doubt that the geology of Tibet was shaped in the Mesozoic by successive collisions with Laurasia of different blocks previously detached from Gondwana[13,21,22], it is not known precisely how and when the different basins of the Tethys opened and closed. Similarly, although the high and apparently recent elevation of the Tibetan plateau must be related to the collision and penetration of India into Asia[23,24], detailed field studies of this large and harsh territory are inadequate for a choice to be made between two extreme models—that of Argand[25], who postulated that Tibet is largely underthrust by the crust of northern India, and that of Dewey and Burke[26], who suggested that Tibet has shortened and thickened more homogeneously (like an accordion) during the late Tertiary.

Our main aim was to improve the understanding of the local geology by using new techniques and observations and to integrate our observations into the framework of plate tectonics.

Sedimentation changes on the Lhasa block

The stratigraphy of the Lhasa region[1,2,7] has been investigated in particular detail near Linzhu (Fig. 1). North and west of Linzhu, the probably Precambrian metamorphic basement (two mica gneisses north of Machu) is covered by Carboniferous tillites (pass north of Linzhu) with Gondwanian affinities. Shallow marine sedimentation dominates from the Permian to the Jurassic: Permian limestones (including reef limestones), intermediate to basic volcanics and pyroclastics (green tuffs and agglomerates), of Lower Middle Triassic age are succeeded by Upper Triassic to Liassic dark-grey limestones with large pelecypods (Megalodontidae).

In the Jurassic, the deposits become more detrital: Upper Jurassic clastic series with coal lenses, clastic formations with large quartz pebbles in the Lower Cretaceous (similar to those in the Himalayas), rhythmic shale–limestone sequence of Upper Aptian age (*Orbitolina* sp.). On top of this sequence, red sandstones and siltstones (Takena formation[1,2]), intercalated with calc-alkaline volcanics and oyster-rich limestone beds, gradually become more and more continental upwards (lacustrine limestone and palaeosols). The Gangdise tonalites or granodiorites (Gangdise plutonic belt) and more recent granite bodies (Lhasa–Yangbajain granites) intrude the folded Mesozoic series which are unconformably overlain by red conglomerates with intercalations of purple volcanic tuffs and thick volcanics (Lingzizong formation[1,2]). The volcanics (chiefly ignimbrites with dacites and andesites) cap most of the high mountains around Lhasa and north of the Linzhu basin and are probably of Palaeocene age[1,2,18].

The stratigraphy agrees well with the inference that the Lhasa block and its extensions to the west and east is a microcontinent

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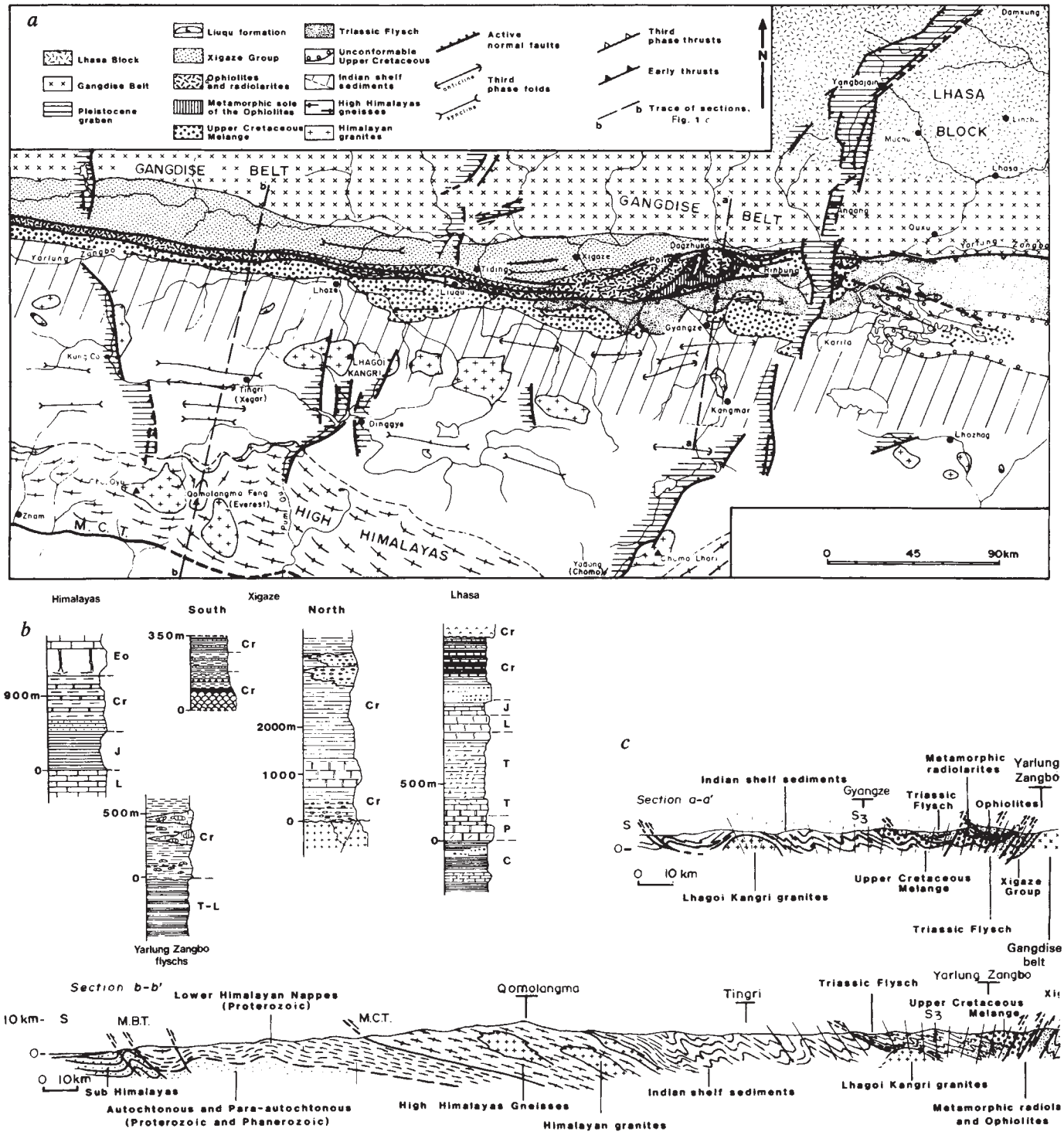

Fig. 1 *a*, Simplified structural map of southeastern Xizang (Tibet) and Himalayas (after ref. 2). Gangdise belt includes mostly granodiorites and granites but also volcanics (ignimbrites and andesites). Triassic flysch is present in the thinly dotted areas and at some places in the area with oblique bars. Oblique bars refer to sediments of the Indian continental slope which have suffered two phases of deformation and schistosities. The thrust contact between these formations and the more autochthonous Indian shelf sediments (one phase of deformation) is not clear everywhere and has not been represented. *b*, Stratigraphic columns of the main elements of southern Xizang (Tibet) (this study and ref. 2). *Himalayan series* (near Tingri): classical succession, from bottom to top: neritic limestones, Lower Jurassic; black shales and marls (Spiti formation), Upper Jurassic; sandstones (Giumal formation), Lower Cretaceous; thin bedded calcschists, with Globotruncanidae, Upper Cretaceous; without any unconformity, thick bedded limestones with abundant microfauna (*Nummulites* sp.), Tertiary (Palaeocene–Eocene). *Zangbo flyschs*: base of column is a flyschoid series, pelecypods (*Daonella* sp., *Halobia* sp.) give a Middle to Upper Triassic age. Top of column is wildflysch (sedimentary mélange), with exotic blocks of various age (Upper Permian, Triassic, Cretaceous), lithology (limestones, cherts and radiolarites, pillow-lavas and so on) and size (cm to km). Globotruncanidae within the matrix give a Campanian (?) Maestrichtian age for this chaotic series which lies in stratigraphic unconformity on top of the Triassic–Jurassic (?) flysch. *Xigaze group*: south—sedimentary stratigraphic cover of the upper pillow-lavas of the ophiolites: red radiolarites and siliceous shales at the base, microfauna (radiolarians) indicate an Upper Albian, Lower Cenomanian (?) age. Rhythmic flyschoid series follow, with locally interbedded calcareous sandstones and coarse breccias (mass flows reworking lavas and cherts); north—the Xigaze group proper in stratigraphic continuity with the cover of the ophiolites but unconformable on Kangdese batholith; rhythmic, thick and coarse-grained series (molassic type). Near Xigaze Airport interbedded levels of highly fossiliferous neritic limestones give an Albo–Aptian age. *Lhasa north-east*: composite column—north of Linzhu for Palaeozoïc basal part and south of Linzhu for Mesozoic upper part. From bottom to top: Tillitoid beds of Upper Carboniferous age; fossiliferous neritic limestones (Permian); thick series of volcanic flows (ignimbrites and so on) and volcano sedimentary beds of Lower(?) to Middle Triassic age; shallow water carbonates with big Lamellibranchiata and corals (Upper Triassic to Liassic); detritic levels with coarse quartz pebbles horizons, locally beds with plant remains and coal seams (Upper Jurassic? to Lower Jurassic); succession of red siltites and fine-grained sandstones with basal level containing *Orbitolina* sp. and oysters (Upper Aptian). Finally, at the top, unconformable volcanic pile of andesites and ignimbrites (late Cretaceous ? Palaeocene). *c*, Cross-sections a–a' and b–b' indicated in *a*. Steep bars (S_3) refer to late cleavage. Thrust contact between Indian shelf sediments and more deformed series to the north on section b–b' is inferred (it corresponds to thin broken line in *a*).

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of Gondwanian origin. In the Lower Mesozoic, it was separated from Laurasia by vast ocean basins, whose traces are to be found along the Kun-Lun, Bang Gong-Nu Jiang and other sutures within Central Tibet to the north[13,15]. It probably detached from Gondwana in the Triassic or a little earlier[22]. Differences in the sedimentary environment north and south of the Zangbo progressively develop from the Triassic onwards. Until the Jurassic, the shallow marine sedimentary facies are still reminiscent of those on the northern margin of India. In contrast, after the Upper Jurassic, the sedimentary facies are more like those found elsewhere along the northern margin of Neo-Tethys, particularly in the Alpine Middle East. This is additional evidence for a large physical break between the Lhasa platform and the Gondwanian 'mainland' during part of the Upper Mesozoic. The most important tectonic deformations probably took place in the late Cretaceous before deposition of the thick upper volcanics.

Oceanic crust and sedimentary cover along Yarlung Zangbo

Considerable work by Chinese geologists has drawn attention to the Yarlung Zangbo ophiolites[1,13-15]. In the region near Xigaze, the ophiolites are particularly well exposed and form an east–west belt ~170 km long and 2–20 km wide (Fig. 1). Most of this belt was mapped during the summer of 1980 at a scale 1/100,000 and three of the largest massifs (Dagzhuka, Xigaze and Liuqu) have been the subject of particularly detailed structural studies (local maps at a scale of 1/25,000; ref. 16) and geochemical sampling.

On the whole, the ophiolites have not been much dismembered or weathered. Except for conjugate strike-slip faults trending ~N 60 °E and 110 °E within the massifs, the major faults are east–west thrusts of the ophiolites over the sedimentary series to the south. Displacements seem to have been a maximum for the Dagzhuka massif which forms a rather large thrust sheet on a sole of metamorphic rocks. Other massifs such as those near Tiding are made of stacked up thrust slices with some retrocharriage to the north onto the Xigaze group. The Xigaze, Liuqu and Polio massifs have merely been tilted ~60° to the north-west and display a remarkably complete and apparently continuous section. A typical cross-section is that of the Xigaze massif (Fig. 2). From north to south (and top to bottom), the southern rim of the Gangdise granodiorite 'batholith' is unconformably covered by variegated conglomerates and molasses representing locally the base of the Xigaze group. To the south the basal clastic sediments appear to grade into pelagic deposits which form the normal sedimentary cover of the oceanic crust[19]. Typically, a few metres of red radiolarian chert encrust the upper pillow-lavas. Above, the cherts give way to a rhythmic brown-green volcaniclastic formation, 300–400 m thick, with radiolaria-rich horizons at the base, of Upper Albian–Lower Cenomanian age.

The polarity of individual pillows (base to the south-east) is consistent with this description. The pillow-lavas are sometimes intercalated with lava flows several metres thick. Under the effusive volcanics, a peculiar succession of dolerite sills with some dyke swarms and exceptional screens of isotropic gabbro are observed. The dolerites, which form a real 'sill complex', are in contact with serpentinized dunites and harzburgites underneath. Further down (south), fresh peridotites consist mainly of harzburgites interbedded with Cr diopside-rich harzburgites. A shear zone of tectonized serpentine containing ophiolitic blocks, marks the basal thrust of the ophiolite complex on the red radiolarian cherts and coarse chert conglomerates.

If not disrupted by internal low angle shears that could have escaped our attention, the 'Xigaze' ophiolites represent a remarkable type of oceanic crust: the crust must have been thinner (at most 3.5 km thick) than the 'normal' crust (~5 km thick) found in many ophiolite complexes around the world. Also, whereas cumulate gabbros are abundant in typical ophiolites, they appear to be almost absent in the Xigaze complex (only small bodies, probably corresponding to magma chambers, have

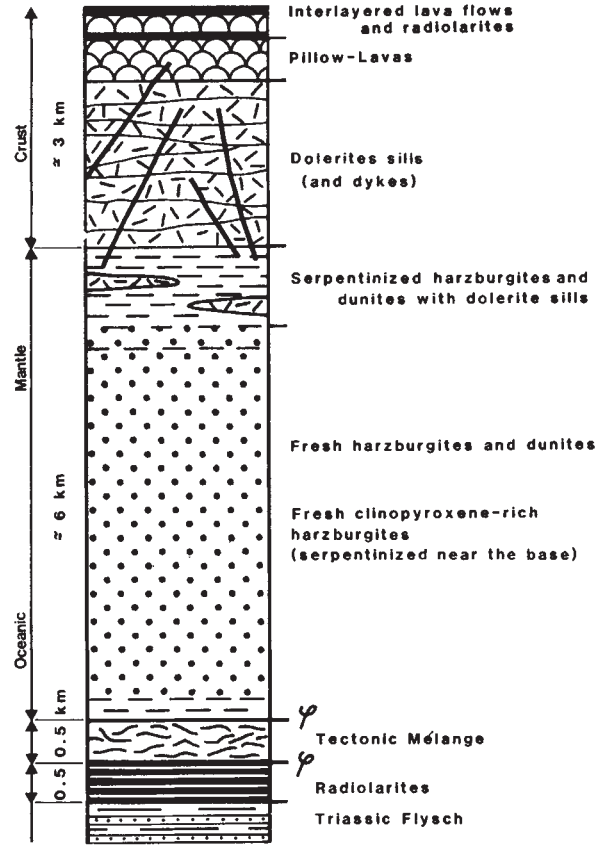

Fig. 2 Simplified section in the ophiolites of the Yarlung Zangbo Suture. (For more details see accompanying article[32].)

been observed in the vicinity of Dagzhuka and Tiding[16]). Finally the existence of Cr diopside-rich harzburgites at shallow depths under the crust is unusual: the ophiolitic oceanic mantle is normally composed of residual harzburgites and the occurrence of lherzolites is rare.

An important discovery is the apparent continuity between the normal pelagic cover of the ophiolites and the more clastic sediments which unconformably overlie the Gangdise granodiorites. This implies that the ophiolites of the Yarlung Zangbo suture zone correspond to the oceanic crust of a rather deep basin (the Xigaze basin), located south of the Trans-Himalayan region but at a short distance from it. Although the age of this basin is not accurately known, the radiolarian cherts above the pillows indicate that it must have formed before the Upper Albian. The main molassic units of the Xigaze group seem to have accumulated mostly during the Upper Aptian[1,2] but perhaps until the Upper Cretaceous and Lower Tertiary.

Triassic flysch and Cretaceous wildflysch

Between Lhozag in the east and Tingri to the west (Fig. 1), the Mesozoic sediments deposited on the north Indian margin are identical to those known as the 'Himalayan' series farther west[27,28] (Fig. 1): black shales ('Spiti') with nodules, of Upper Jurassic and Lower Cretaceous age, Lower Cretaceous sandstones and quartz pebbles conglomerates, Upper Cretaceous white limestones and mudstones with *Globotruncana*, and nummulitic limestones (Middle–Upper Eocene). There is no unconformity between the Upper Cretaceous and the Tertiary, which are merely separated by a hard ground in the mountains west of Tingri. Between the thick and monotonous deposits of the Indian margin and the ophiolites of the Zangbo suture, two distinct flysch formations of different ages and nature must be separated: the Triassic flysch and the Upper Cretaceous wildflysch or mélange.

The *Daonella*-bearing flysch is most likely to be of Triassic to Liassic age and slightly metamorphic (sericite). Facies of the rhythmic sediments appear to be quite similar to those of the Lamayuru flysch recognized in Ladakh[22,27,28]. On the other

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hand, the mélange formation, which was long assimilated to the Triassic flysch, is not metamorphic and is best described as a dissociated wildflysch with exotic blocks. The blocks are of various sizes (from a few centimetres to a few kilometres), ages and facies and are sometimes more deformed than the matrix.

The most common elements are: Permian (Djulfian) white-grey limestones with crinoids and foraminifera (*Agathammina pusilla* Geinitz, *Geinitzina postcarbonica* Spandel, *Calcitornella heathi* Cushman and Waters, *Stipulina* sp. Lys), Lower Triassic pink limestones (*Meekoceras* sp.) of 'Himalayan facies', Lower Jurassic (?) 'filament' limestones, Campanian–Maastrichtian limestones (*Globotruncana*), blocks made of a primary association of pillow-lavas and red or green micrites, grey, red or green cherts and grey chert breccia. Some blocks are composite: fragments of Permian, Triassic and Jurassic rocks are cemented by micritic limestones of Campanian–Maastrichtian age (*Globotruncana stuarti* or *arca* (Fig. 3). The largest (kilometre size) olistolithes are primarily formed of fossiliferous Permian limestones and marl limestone rhythmites or pelagic unmetamorphosed Triassic–Liassic flysch. The matrix is a rhythmic sediment with *Globotruncana*-bearing carbonate beds.

The wildflysch or mélange thus should be at least of uppermost Cretaceous age. Whereas the Triassic flysch probably corresponds to the accumulation prism along the passive northern margin of the Indian continent, the Cretaceous wildflysch undoubtedly formed in a more active tectonic environment, perhaps in the accretion prism of the subduction zone along the active margin of Asia.

Overthrusting and shortening north of the Himalayas

In many places, the ophiolite belt along the Yarlung Zangbo seems to be limited to the north and south by steeply dipping faults. More detailed observations show that these faults correspond to rather minor and late tectonic events. The most important deformations and displacements have occurred during earlier phases responsible for the large scale overthrusting of the ophiolites to the south. Microtectonic studies permit the identification of four principal phases of shortening, the first and the third being the major ones.

The most intense deformations seem to have taken place during the first phase (φ_1). They are best observed in the metamorphic sole under the ophiolites. Both the radiolarian cherts and associated pelagic limestones (marbles) and the schists with thin chert, sandstone and metatuff beds underneath show subhorizontal cleavage and a strong stretching lineation striking N 150° to N 180 °E. As the cleavage is parallel to the basal thrust, and the lineation parallel to the apparent direction of transport, the deformation and metamorphism probably occurred during overthrusting. The Triassic flysch is also affected by an early deformation phase with flattish flow cleavage and roughly north–south stretching lineation.

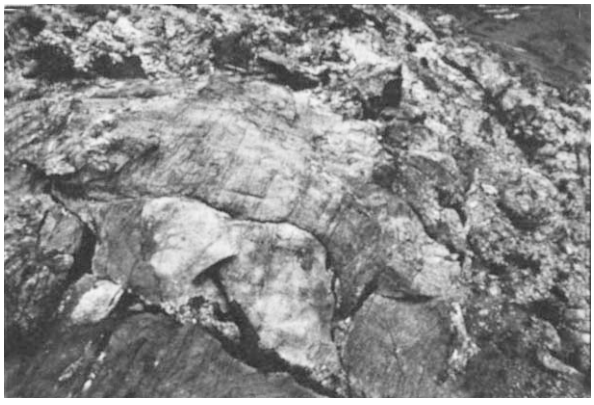

Fig. 3 Close-up of a Triassic block included in a primary Upper Cretaceous mudstone matrix; the whole is reworked as an exotic block in the late Cretaceous wildflysch (~20 km south of Xigaze).

Kilometric size folds overturned to the south or west can be related to this first phase.

The second tectonic phase (φ_2) is still characterized by north dipping thrusts. However, there seems to be no penetrative deformation and no metamorphism associated with this phase. The final effect is an exaggeration of the early overthrusts which brings the ophiolites, the metamorphic cherts and the Triassic flyschs onto the mélange and sometimes the autochthonous series of the continental slope of India.

The pronounced south vergence of the earliest tectonic events in the Yarlung Zangbo zone implies that some of the oceanic crust of the Tethyan Sea was subducted to the north under the Xigaze basin. The deformed cherts and pelagic slates now incorporated in the metamorphic sole of the ophiolites may represent scraped fragments of the late-Jurassic to mid-Cretaceous cover[7] of this crust. However, because the strong deformation linked with the large scale overthrusts also affects the Triassic flysch and the autochthonous series, and it is logical to relate most of the tectonics to the emplacement (obduction) of the ophiolites onto the northern margin of India.

Along the Yarlung Zangbo zone, the Upper Cretaceous mélange, the Triassic flysch and autochthonous Indian series, and the thrust contacts have been folded again by a third phase (φ_3) characterized by upright folds with undecided vergence and fan-shaped cleavage (S_3) (Fig. 1). The third phase is especially clear in the region near Rinbung where it has produced retrocharriages: slices of ophiolites, radiolarian cherts and Triassic flysch have been emplaced onto the Xigaze group, along south dipping thrusts outlined by fragments of serpentine. In the series of the Indian margin, south of the granite batholiths of the Lhagoi Kangri range, the third phase is the only one to be observed in the Eocene (near Tingri). The intensity of the deformation and the asymmetry of the folds related to this phase increase towards the south into the lower units of the 'High Himalaya slab'. In the Cambro–Ordovician, the folds are clearly south vergent and the flow cleavage synchronous with metamorphism. Yet further down and south, ductile shear becomes predominant in the augen gneisses injected with tourmaline leucogranite sheets parallel to the foliation. The third phase is also essentially responsible for the deformation of the sediments of the Xigaze group whose uppermost units are perhaps of Eocene age. The style of folding is typical of late phases of rather symmetrical shortening: east–west folds, with steeply dipping cleavage (Fig. 4) are slightly south vergent in most of the basin (subvertical southern limbs). The vergence to the south is most pronounced near the southern edge whereas a north vergence appears locally near the Gangdise batholith (Fig. 1).

According to some of us (P.M., J.A., J.P.B. and M.B.) three independent sets of observations suggest that the early deformations synchronous with the emplacement of the ultrabasic thrust sheets (φ_1) have occurred before the deposition of the mélange[20]: (1) between Gyangze and Lhaze, the mélange or wildflysch overlies unconformably the Spiti series; (2) whereas two phases of deformation (φ_1 and φ_3) are observed in the Spiti sediments, only one phase (φ_3?) can be recognized in the mélange; (3) elements in the mélange have experienced metamorphic deformation (perhaps φ_1) before their incorporation in the mélange's matrix which displays only φ_3 style deformations.

The most recent structures that can be attributed to north–south shortening in southern Tibet are discrete reverse faults, dipping rather steeply to the north or south. In several places (near Tiding, for example), the faults clearly cut folds of the third phase, and thus belong to a distinct tectonic event, here referred to as phase four (φ_4). This fourth phase is rather modest and does not manifest itself by distributed folding of the series. It is responsible for the accentuation of the retrocharriage of the ophiolites and cherts slices onto the Xigaze group, which obliterates in most places the root zone of the ultramafic nappes.

In the region of Lhasa, Yangbajain and Linzhu, north of the Gangdise granodiorites, the most prominent folding of the

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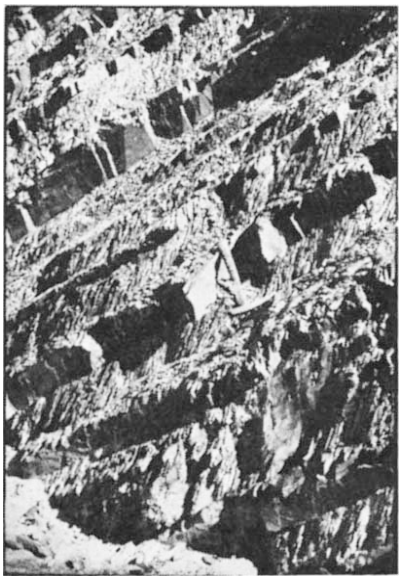

Fig. 4 Well developed steeply dipping slaty cleavage S_3 in the Xigaze rhythmites, 1 km west of Tiding.

Mesozoic sediments, also with steeply dipping cleavage and undecided vergence, has occurred before the Palaeocene: precise $^{40}Ar/^{39}Ar$ dating of the upper volcanics (Lingzizong formation) which lie unconformably on the folded continental Cretaceous red beds (Takena formation), but are not much deformed themselves, yielded an age of 60 Myr (ref. 18). The Lingzizong formation is only gently folded and broken by several steeply dipping east–west reverse faults, which can be attributed to the fourth phase of compression identified in the south. Perhaps the most prominent of these faults is that which bounds the Linzhu basin to the north: the fault clearly post-dates the Palaeocene ignimbrites and andesites of the Lingzizong formation which are offset several hundred metres.

Still later in the Tertiary, strike-slip faulting may have occurred, especially in the Zangbo suture zone. We found good microtectonic evidence for right lateral strike-slip faults, striking 110°–120° N, south of Quxu. There, the suture appears to be more linear and vertical than in the region of Xigaze, a character often indicative of important strike-slip movements. The most recent deformations in Tibet, however, do not correspond to north–south shortening but, perhaps surprisingly, to east–west extension of the crust.

Tibetan plateau extension during the Quaternary

Although some north–south faults in Tibet had already been described[2,13], it was the Landsat images[24,29] that first suggested that normal faulting and active east–west extension was a dominant tectonic process over most of the plateau. This inference was supported by fault plane solutions of earthquakes. However, perhaps because extension seemed to contradict prevailing views on how the plateau formed[26], many have been reluctant to accept it in the absence of more abundant and direct field evidence. We now have an ample collection of field observations[17] which demonstrate that recent distributed normal faulting is as important in southern Tibet as it is in regions such as the Aegean or the Basin and Range. The observations also show how Landsat photos can be used to study active tectonics.

Several normal fault systems cross our study area: from west to east, the most important ones are the Kung Co system, immediately north of Cho Oyu and Qomolangma Feng (Mt Everest) and the Yadong–Karila–Angang–Yangbajain–Damxung–Gulu system, which extends some 600 km from Chomo-Lhari to the south, to the region west of Amdo in the north (Fig. 1) (see also the accompanying paper[30]). We were able to map in detail some segments of the Yadong–Gulu graben using aerial photographs[17].

The faults are especially clear in the morphology (Fig. 5). They cut moraines and post-glacial Quaternary terraces in many places (such as Angang, Damxung and Kung Co). In some places glacial valleys have been offset several hundreds of metres, which gives an order of magnitude for cumulated vertical movements since the end of the glaciation. Although most of the faults trend north–south, oblique faults cut or connect them. We found evidence for conjugate strike-slip motion along them.

Five clusters of recent surface breaks have been found west and north of Yangbajain. Uprooted bushes and disrupted turf suggest that most of these have been caused by earthquakes that occurred in the past few decades. The most ancient ones cannot be much older than a couple of centuries. All the breaks reactivate faults clearly visible on the satellite images. Particularly recent breaks are those which run along the western fault of the Gulu graben, south of Gulu, with ~3 m of normal slip, and that found south of Lake Peng Co, with ~5 m of right lateral strike-slip movement which we relate to the large earthquake of 18 November 1951 ($M = 8$) (see accompanying paper[30]).

Many microtectonic measurements were taken in different favourable sites, either in 'basement' rocks near the master faults (Angang, Damxung and Kung Co) or within the Quaternary gravel beds of terraces (Yangbajain, Angang). Although the data are still being processed, preliminary results are consistent with a roughly east–west minimum principal stress (σ_3) and a vertical maximum principal stress (σ_1). This stress state should rule out Quaternary folding or thrust faulting, for which we have found no field evidence.

Although no Quaternary volcanics were observed in the region we surveyed, hot springs were numerous and widespread. The rise of hot waters through the crust seems to be a direct consequence of east–west extension because the water often finds access to the surface through north–south cracks. This was particularly clear south of Kung Co, where travertine fissure systems have been mapped in detail.

Conclusion

The present field work has improved our knowledge of the geology and tectonic history of southern Tibet. Although it substantiates earlier conclusions[1,2,7,13–15,21,24], it also raises important questions and helps to define priorities for future field research.

It is now established that the Zangbo ophiolites are structurally attached to the Lhasa block and Gangdise calc-alkaline plutonic belt. This supports the idea that the Gangdise belt is the product of northwards subduction of the floor of Neotethys and that the Xigaze basin might have been a fore-arc basin[7]. It is also likely that the 'Lhasa block' is a fragment of Gondwana, but when it became attached to Asia is unknown. For example, we do not know whether the Gangdise batholith was emplaced along an Andean margin, or along an island arc containing some continental fragments. Although great changes can occur along strike, the Ladakh–Kohistan batholith, which is the analogue of

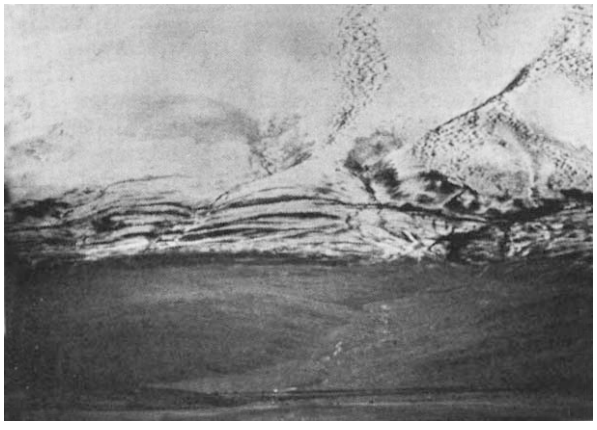

Fig. 5 Spectacular Quaternary scarps, outlined by snow, along the western master fault of the Gulu Graben (north–south trending faults).

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the Gangdise batholith in the western Himalayas, has been interpreted as such an island arc in the Mesozoic[31].

The timing of tectonic deformations also raises problems. For instance, the age of emplacement of the Zangbo ophiolites onto the northern margin of the Indian continent is not yet well established. Some of us think it might have occurred earlier than at the end of the Eocene, which would place new constraints on speculations based on plate tectonic reconstructions.

Perhaps one of the most surprising observations was the comparatively small amount of shortening and deformation of the 'surface' sediments, north of the Zangbo ophiolites. Upright folds with steep cleavage predominate and no large-scale tangential tectonics seems to have occurred in the Upper Mesozoic series. Folding and deformation has been especially mila in the Tertiary series around Lhasa and farther north. This is in sharp contrast to the huge thrusts in the lower Himalayas, or even typical amounts of shortening in other large orogenic belts and may be related to the flat topography of the Tibetan plateau over most of its surface. If such modest shortening in the Tertiary also prevails far north and west of the regions described here, India's northward push may have elevated Tibet in a different way than accordion shortening and thickening of the crust[26].

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Field evidence for active normal faulting in Tibet

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Crustal thickening does not now occur in southern Tibet. Field observations made during the Chinese–French expedition of 1980 support an earlier hypothesis based on a combined analysis of satellite images and fault plane solutions of earthquakes. Immediately north of the highest peaks of the Himalayas, the tectonic regime is dominated by east–west extension. We have mapped a large number of north–south normal faults which sharply cut the glacial and post-glacial morphology, and followed several recent earthquake breaks along faults detected on satellite and aerial photographs. Microtectonic measurements in the Quaternary suggest that the maximum principal stress is vertical.

TECTONIC extension sometimes occurs far within continental regions, with no obvious connection with the world rift system and thus the causes are poorly understood. Of all these regions, Tibet is perhaps the most surprising. Since Argand presented his ideas on the tectonics of Asia[1] Tibet has emerged as a symbol of continental collision. Today, within the framework of plate tectonics, there is little doubt that the massive plateau, which maintains an elevation of some 4 km, over 1,000 km north of the Himalayas, is anything but a direct consequence of the most spectacular collision in the Cenozoic—that of India with Eurasia. However, the forces that have pushed up this immense region and somehow thickened its continental crust[1–3] do not thicken it appreciably now. Instead, the crust of Tibet is stretched in an east–west direction. The first field observations of the 1980 French–Chinese expedition firmly establish predictions made with satellite images and fault plane solutions of earthquakes[4,5] and substantiate descriptions already made by Chinese geologists both from aerial photographs and in the field[6]. Here we summarize the most spectacular evidence for recent normal faulting and the ensuing preliminary conclusions.

Large normal fault systems of southeastern Tibet

As can be seen on Fig. 1, large normal faults cut across the middle reaches of the Yarlung Zangbo River. Figure 1 shows a refined version of earlier maps prepared from satellite images only[5]. Because our field survey was not exhaustive, the present document has also been based on a more detailed interpretation of enlarged and/or better quality Landsat images of the region. However, as we found good correspondence between faults previously recognized on the images and Quaternary faults that could actually be observed in the field, we believe that Fig. 1 represents fairly accurately the pattern of active tectonics in southeastern Tibet.

Between 86° and 91 °E, the only area within the limits ascribed to the 1980 French–Chinese study, the normal faults group into three major systems, elongated in a roughly north–south direction.

Along most of the length of the three rift systems one master fault on one side of the flat Quaternary floor is usually more

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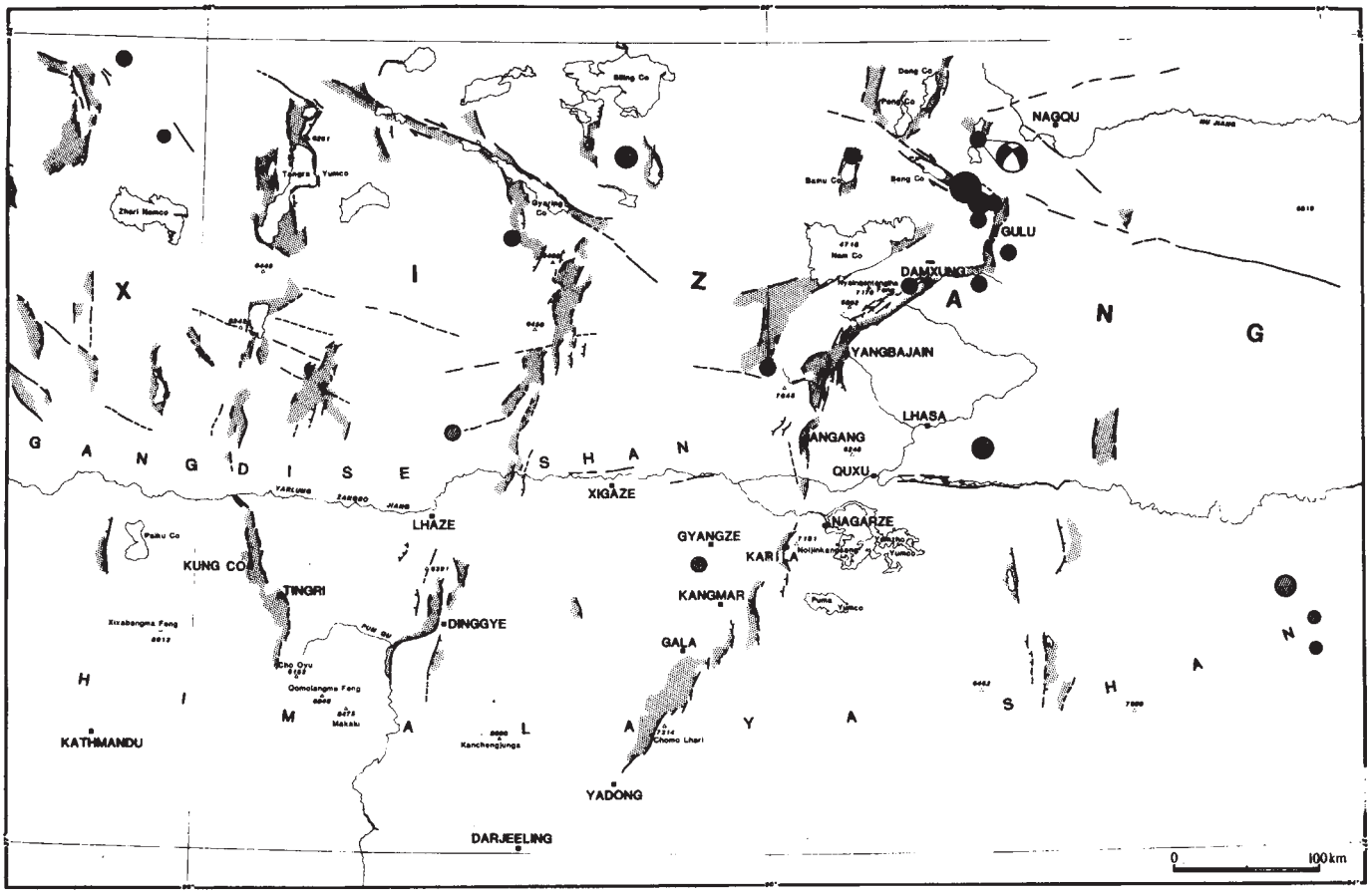

Fig. 1 Map of active tectonics and seismicity of southeastern Xizang and surrounding regions. Earthquake epicentres are from the map of epicentres of strong shocks of China[9,10]. Sizes of circles correspond to magnitudes (small, $6 \leq M < 7$; intermediate, $7 \leq M < 8$; large, $8 \leq M$). Fault plane solution is from ref. 5.

prominent than the other. Sometimes, there is only one such fault, as may also be observed in Greece, western Turkey and the Basin and Range. In addition the faults are discontinuous along strike, often forming *en échelon* arrays. The most prominent of the three systems is located to the east (Figs 1 and 2*a*). It extends ~600 km from Yadong, at the foot of the Chomo Lhari range, across the Zangbo suture and the Gangdise granodiorites, all the way to the headwaters of the Nu Jiang (Salween river). Because it is close to Lhasa, and along a major communication axis, we studied it more closely than the others, especially in its central part between the Zangbo and Gulu (Fig. 2*b*). Abrupt elevation differences along the main faults are typically of the order of 2 km. The three highest peaks of the region are located along the uplifted shoulder of the rift: they rise to >7 km (for example, Mt Noijinkangsang, Mt Nyainqentanglha) (Fig. 1). To the west, the Pum Qu and Kung Co Fault systems (Figs 1 and 2*c*) were less accessible. In particular, it was not possible to reach regions north of the Zangbo, and the Pum Qu faults between Tingri and Dinggye were seen only during a 2-day reconnaissance trip. Nevertheless, preliminary observations confirmed that the faults are active. The two fault systems are quite different: whereas the faults near Kung Co form a long and linear half graben, those that cut the Pum Qu valley make a cluster of more or less parallel breaks whose continuity across the Zangbo suture zone with the faults that extend northwards to Gyaring Co is not well expressed in the topography.

Evidence for post-glacial faulting

In many places along all three 'graben' systems, post-glacial faulting offers spectacular sights. The most numerous and clear examples are found along the Yadong–Gulu 'graben'. We observed three types of morphological evidence.

(1) Quaternary slopes with their ridge and gully topography are sharply cut by the faults (Fig. 3*a*) along most of their lengths, with typical offsets of a few to a few tens of metres.

(2) Near Damxung and Gulu (Fig. 1) attention is caught by several hanging fluvial valleys: ancient valley floors, or flat alluvial fans of tributaries are elevated several metres to a few tens of metres above the present day level of the Lhasa river valley.

(3) West and north of Yangbajain, the same process, although presumably on a longer time scale, has produced perched glacial valleys (Fig. 3*b*). This is the most impressive effect of Quaternary normal faulting as valley floors and lateral moraines are sometimes offset as much as several hundred metres.

South-west of Yangbajain (Fig. 1), the Quaternary floor of the Yadong–Gulu 'graben' is cut by a remarkably large number of roughly north–south trending scarps, especially on its eastern side (Figs 1, 2*b* and 4*a*). The resulting rift in rift structure, with outward tilt of the Quaternary terraces, is reminiscent of regions of Afar or Iceland, if only because of the fault density. This area will be described in more detail elsewhere[7,8].

Post-glacial normal faulting is also found along some of the other rifts. For example, spectacularly uplifted glacial valleys, also visible on the satellite images (Fig. 2*c*), testify for some 500 m of vertical displacement along the master fault near Kung Co, presumably in the past several thousand years. Clearly, more detailed and systematic studies of the post-glacial morphology will be useful for estimating recent extension rates in southern Tibet.

Historic surface breaks

Several surface breaks that can be attributed to recent earthquakes were found along the Yadong–Gulu graben, between the Zangbo and Peng Co (Lake Peng) (Figs 2*b*, 3*a*, 3*c*). All of these reactivate faults that cut the Quaternary morphology, or are located near such faults. Typical vertical offsets are of the order of a few metres. The most recent breaks are observed west of Yangbajain, near Gulu, and near the southern tip of Peng Co: there, the disrupted vegetation has not grown back and the

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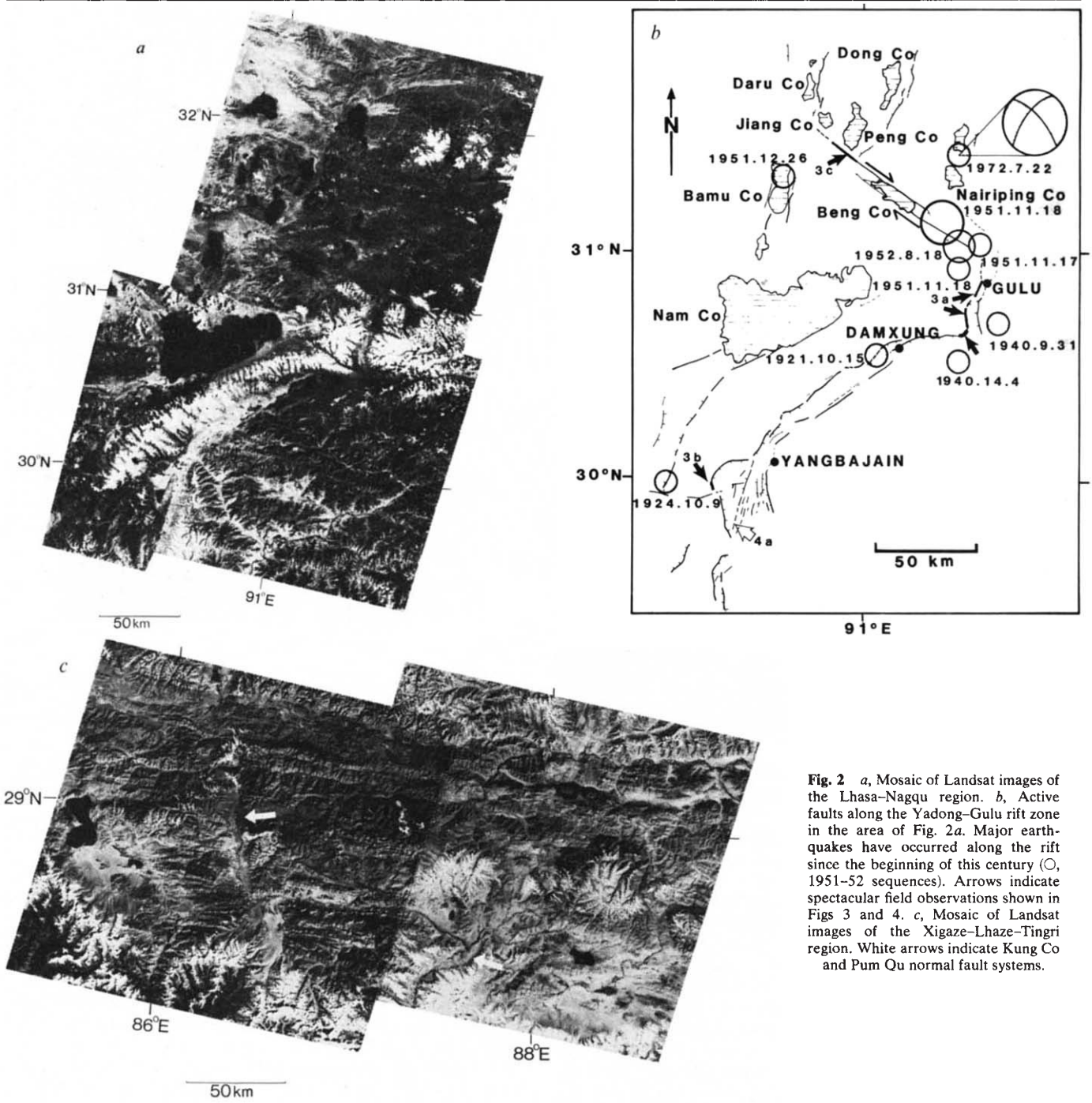

Fig. 2 *a*, Mosaic of Landsat images of the Lhasa–Nagqu region. *b*, Active faults along the Yadong–Gulu rift zone in the area of Fig. 2*a*. Major earthquakes have occurred along the rift since the beginning of this century (○, 1951–52 sequences). Arrows indicate spectacular field observations shown in Figs 3 and 4. *c*, Mosaic of Landsat images of the Xigaze–Lhaze–Tingri region. White arrows indicate Kung Co and Pum Qu normal fault systems.

corresponding earthquakes probably occurred in the past few decades. Epicentres on Figs 1 and 2*b* are from the historic catalogue of earthquakes compiled by the Institute of Geophysics of the Academia Sinica[9,10]. As for most of Western China, the catalogue contains only shocks which have occurred since the turn of the century, a fact that reflects the low density of population. In addition, we do not know the accuracy with which the epicentres are located, and this makes correlations with surface breaks difficult. Nevertheless, it is tempting to associate the freshest ground breaks near Gulu and Peng Co with the major earthquake sequence that shook the region in 1951–1952.

The sequence, the most important known to have taken place within the Tibetan plateau, was composed of one magnitude 8 shock, one magnitude 7 shock, and at least two shocks with magnitudes >6. All the epicentres cluster near the eastern extremity of a long and linear fault zone (Fig. 2*a*, *b*), which we will refer to as the Beng Co fault. This tectonic feature, particularly sharp on the satellite images, has been interpreted as a predominantly strike-slip fault[5,11]. One of the major finds of a reconnaissance trip to the region of Peng Co, which was more easily accessible, was a straight alignment of pressure ridges and cracks (mole tracks) in the meadows immediately south of the lake (Fig. 3*c*). In two places, the rather fresh break, oriented ~N 135° E, cut across Quaternary ground morphology and we could estimate a right lateral offset of at least 5 m. This supports the idea that the Beng Co fault is indeed essentially a right lateral strike-slip fault and that it was activated by the magnitude 8 earthquake of 1951. Although the epicentre is located near the eastern extremity of the fault, the break appears to have propagated at least 100 km to the west, to the place where we observed it. The main shock of the 1951–52 sequence would thus be strike-slip but it is not unlikely that foreshocks and aftershocks (Figs 1, 2*b*) have ruptured the normal faults of the Gulu graben, (Figs 2*b*, 3*a*). According to one of us (H.T.), the study of annual growth rings of trees on both sides of the normal fault south of Gulu supports the inference that this fault was activated in 1951–52.

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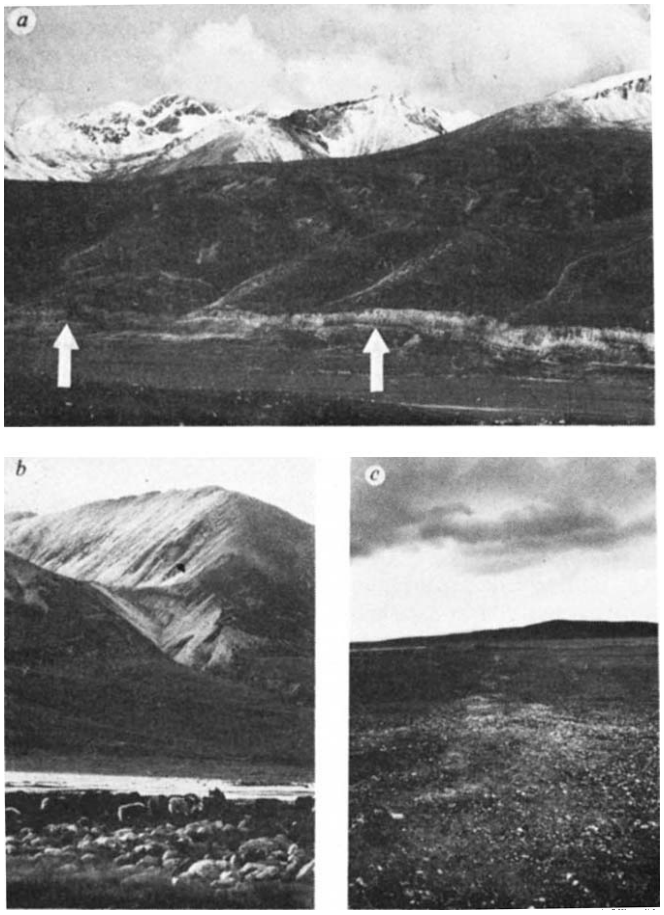
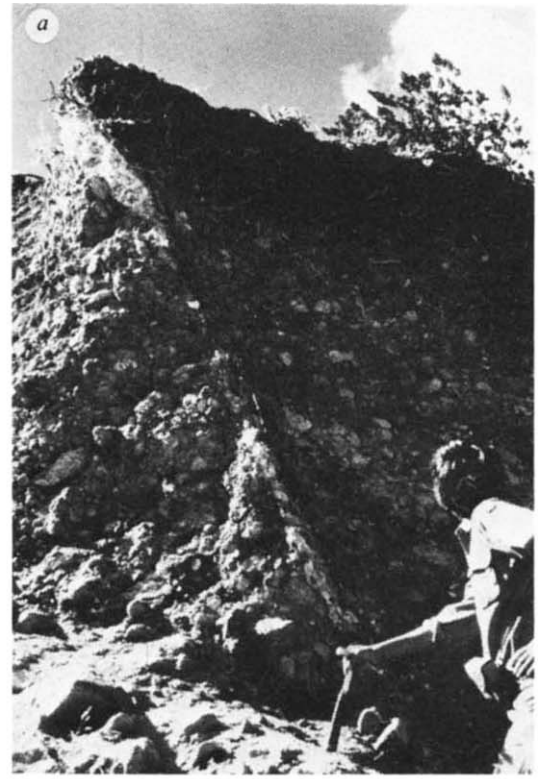


Fig. 3 *a*, Quaternary normal fault near Gulu. The thin white line (white arrows) which runs along the main scarp probably corresponds to the surface break of one of the 1951–52 earthquakes. *b*, West of Yangbajain, glacial valley and moraines are cut by a prominent normal fault. *c*, Eroded mole tracks follow the straight surface break presumed to be that of the magnitude 8 earthquake of 18 November 1951.

Geometry of faulting and stress in the crust

The observations above (Figs 1, 5) show that the present tectonic regime in southeastern Tibet is dominated by east–west extension. However, as was already suspected from the satellite images, this involves not only north–south trending normal faults, but also strike-slip faults, which sometimes provide the kinematic link between distinct normal fault systems, much as transform faults between ridge segments in the oceans. Such is the case for the Beng Co fault, and probably other faults with similar orientations (for example along Gyaring Co, Fig. 1). Faults with a 'conjugate' strike (N 60° E) also exist and we found good evidence for left lateral movements along them (see Fig. 1, near Yangbajain and Damxung)[7]. In addition, the *en échelon* geometry of some normal fault systems (Fig. 1) favour strike-slip components in the deformation of the crust.

These kinematics are born out by our microtectonic measurements either in the Quaternary (Fig. 4*a*) or in the fractured bedrock and mylonites near the main faults. Although these measurements and the inferences from them will be reported in detail elsewhere[8], a sample diagram is shown in Fig. 4*b*. Orientations of the faults are variable, and the corresponding slip on them entails a variable strike-slip component. Nevertheless, all faults have a large normal component and are compatible only with extensional tectonics. Nowhere did we observe reverse faulting or folding in the Quaternary. The extension direction can change from one site to the next, but it is east–west on average. Numerical inversion of the microtectonic data (slickensides)[12] is consistent with a vertical maximum principal stress (σ_1) and a roughly east–west minimum principal stress (σ_3) (ref. 8).



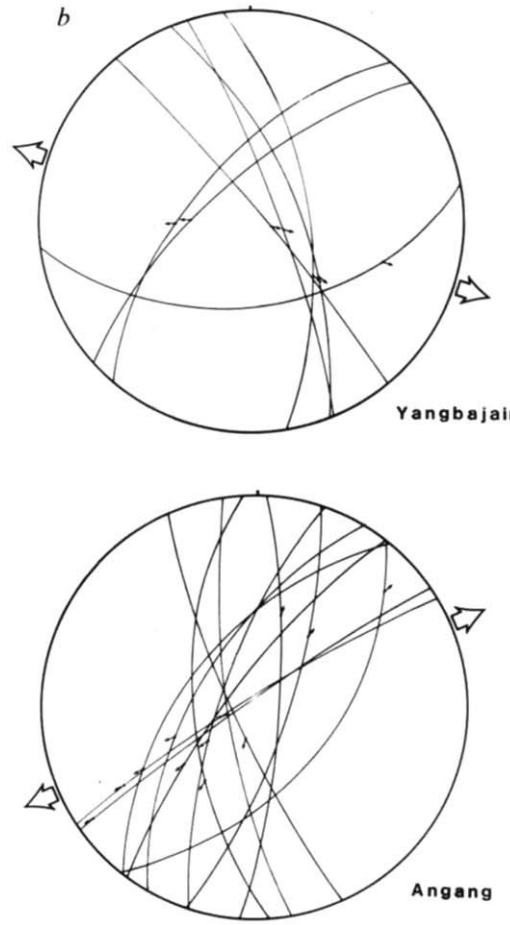


Fig. 4 *a*, South of Yangbajain: normal fault cutting Quaternary terrace gravels. Many other normal faults occur nearby. *b*, Measurements of slickensides (arrows) on Quaternary faults (great circles) in the regions of Angang and Yangbajain (lower hemisphere projection) indicate roughly east–west extension.

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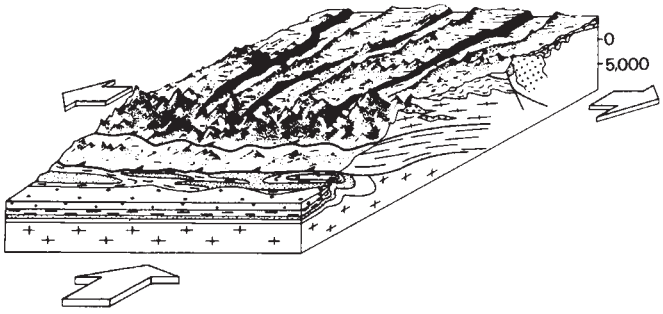

Fig. 5 Idealized block diagram shows how, whereas folding and shortening of the crust still occurs in the Siwaliks and lower Himalayas in response to the northward penetration of India into Asia, east–west extension now predominates on the high Tibetan plateau.

Conclusions

Although north–south shortening of the crust is evidenced by upright folds and cleavage in early to middle Cretaceous sediments and much more gentle folding in late Cretaceous and early Tertiary sediments[6,13,14], shortening has apparently not been a dominant process in the late Cenozoic tectonics of southern Tibet. During the upper Quaternary at least, but perhaps for a longer period of time, east–west extension must have prevailed on the Tibetan plateau. North–south normal faulting has been especially active in the past several thousand years. As in other areas such as the Aegean in the Mediterranean, or the Basin and Range in North America, such faulting appears to be distributed over a wide area.

The faults are perpendicular not only to the Himalayan belt in which they stop rather abruptly, but to most of the more ancient Mesozoic and Tertiary fold trends and roughly east–west structural and palaeogeographical zones within the plateau. In particular, the fault systems cut through major sutures such as the Yarlung Zangbo. Thus, although deviation by more ancient structural trends is possible (Fig. 1), a large proportion of the north–south normal faults are probably not reactivated older ones as has often been suggested[15]. Hence, perhaps more than in any other region, extension in Tibet seems to reflect a particular state of stress in the crust and upper mantle, where gravity can be the main driving force. Microtectonic observations made during the Chinese–French expedition support this inference.

Although further speculations on the cause of the east–west extension must await a better knowledge of the upper mantle and crustal structure of Tibet, the approximately double thickness of the continental crust as well as the softness of the hot lower crust[16] probably favour east–west flow in response to India's northward push[5,17].

Now that widespread normal faulting on the Tibetan plateau can be considered more firmly established, important questions remain for future field work: when did incipient extension take over folding and crustal thickening? Does the time when this change occurred compare with the time when Tibet acquired its abnormally high elevation? At what rates has east–west stretching of the crust taken place since then?

New data on these questions will surely place constraints on how large and high plateaus form and evolve during continental collision.

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The Xigaze ophiolite (Tibet): a peculiar oceanic lithosphere

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The Xigaze ophiolite which outcrops along the Yarlung Zangbo river, southern Tibet, locally displays a complete ophiolitic sequence from marine sedimentary cover over basaltic volcanics to the north, to fresh Cr diopside-rich harzburgites to the south. In contrast with other ophiolites, the mafic part of the sequence is particularly thin. It is almost devoid of cumulate gabbros consisting of a diabase sill complex covered by lava-flows or pillow-lavas. The ultramafic unit is poor in residual harzburgites and dunites and consists dominantly of fresh Cr diopside-rich harzburgites. The origin of this peculiar ophiolite (of oceanic crust) is discussed.

OPHIOLITE complexes in various parts of the world display remarkably comparable sections with, from top to bottom, basaltic lavas, dolerite dykes, isotropic gabbros, cumulate gabbros and ultramafic rocks, resting on tectonic dunites and harzburgites. The mafic part of these complexes usually represents a thickness of between 3 and 6 km (refs 1,2). It is this remarkable homogeneity, at least in their gross features, which led to the concept of ophiolites and to its informal definition[3]. In contrast to this homogeneity, various oceanic environments have been considered as potential sites for the origin of ophiolites: oceanic ridges with various spreading rates[4–10], back-arc basins[11,12], island arcs[13,14] and even transform faults[15]. The Xigaze ophiolite outcrops in Tibet along the Indus–Zangbo suture zone, one of many sutures which separate the Indian continent from the northern plate(s). Only limited data are available about these ophiolites[8,10] although they have been discussed in a recent report[16] that draws attention to the presence of limited amounts of cumulate gabbros and suggests that they may well correspond

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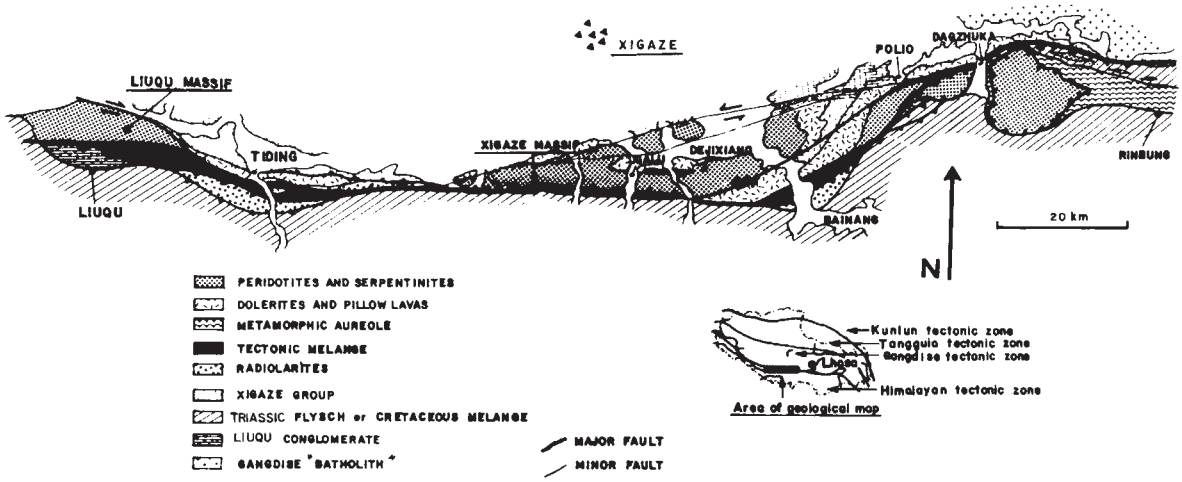

Fig. 1 Geological map of the Yarlung Zangbo ophiolite belt around Xigaze.

to a slow-spreading ridge on the evidence of a "thin, perhaps discontinuous plutonic sequence".

Considering that, as far as we know, ophiolites without cumulate gabbros have been identified only in highly dismembered massifs, the detailed structural study of the Xigaze ophiolite was of particular interest. It should contribute to the discussion on the relation between the existence of magma chambers in the oceanic crust beneath spreading centres and the problems of rate of partial melting in the mantle and rate of oceanic expansion.

The Xigaze ophiolite

The Indus–Zangbo suture in Tibet is commonly marked by a discontinuous line of serpentinites or by minor ophiolite bodies. The Xigaze ophiolite belt is >170 km long in the east–west direction of the suture and has a maximum width of 20 km (Fig. 1). It contains three areas where large masses of ultramafic and mafic rocks have been preserved from the intense deformations observed elsewhere along the suture zone: the Dagzhuka, the Xigaze and the Liuqu massifs. In the Dagzhuka and Xigaze massifs, a sequence from sedimentary cover over basaltic volcanics to the north to fresh harzburgites rich in Cr diopside to the south has been identified. Together with more partial sections such as those of Dejixiang, Polio and Tiding they enable a schematic cross-section to be constructed for the Xigaze ophiolite (Fig. 2).

Due to collision along the Indus–Zangbo suture zone, the ophiolites in the Xigaze area have been tilted into a 060°, 60°NW attitude with only local variations. As a result, the top of the sequence and its sedimentary cover are located to the north of the section. Generally, the main lithological contacts in the ophiolite, oriented at 060°, 60°NW, are few tectonized. They contrast with the east–west contacts, the main one being the southern limit of the ophiolite, which cut the former and are systematically tectonic.

From north to south (Fig. 2) one first meets the Gangdise 'batholith' which is commonly ascribed to an island arc or a continental active margin environment[13,18]. Its location relative to the suture, its east–west orientation and its lithologies point to the presence of an underlying, northward subduction zone. The northern part of the Xigaze group is considered as an essentially detrital formation accumulated on the southern margin of the granodiorite (Gangdise) massif. Its contact with the ophiolite often is a stratigraphic one with a few metres of cherts over the basaltic volcanics, covered by marine pelagic sediments reworking in various proportions the underlying volcanics[19].

Below the cherts either pillow-lavas or lava-flows are observed (Fig. 3). They are parallel to the chert orientation with local departures in the pillow-lavas. The polarity in the pillow-lavas is always with the top northward. Their diameter in section is smaller than 60–80 cm and they are often extended in tubes with no particular directions. They are either largely variolitic, with 1-cm varioles or massive with only a finer grain rim. Their matrix is not abundant and exclusively constituted by volcanic debris and glass. Beneath a few hundred metres of volcanics, the first diabase dykes and sills appear. They typically present chilled margins and have been observed cutting through the pillows. They progressively become more abundant until they constitute a sill complex with sills intrusive into sills. The sill rather than dyke nature is deduced from their general parallelism with the overlying volcanic lava-flows and sediments. A few dykes, normal to the sills, are often present (Fig. 3); these can be very abundant in some massifs such as Dagzhuka. The thickness of individual sills is in the metre range. When they reach a few metres, microgabbro textures can be obtained in their core. New sills intrusive in such rocks can create the illusion that they are intrusive in a fine-grained gabbroic formation. True plutonic rocks are uncommon between diabase sills with the exception of a few isotropic gabbros and trondjhemite screens.

In the Dagzhuka and Tiding massifs (Fig. 1), small bodies of cumulate gabbros and ultramafic rocks are locally present at the base of this sill complex. In these massifs, the thickness of the cumulate olivine gabbros is <170 m and <120 m respectively. In the Dagzhuka massif, the walls of the chambers are made of troctolites and dunites which can be interpreted as the first cumulates or, alternatively, as residual wells impregnated by feldspar coming from the chamber. The shape of the magma chambers is very irregular and, in Dagzhuka, the total thickness mentioned above could be achieved by addition of smaller chambers. In Tiding, there is a remarkably thick sequence of isotropic gabbros which may represent the upper part of a comparatively large magma chamber.

The thickness of this crustal unit does not exceed 3 km. It lies over a serpentinized harzburgite and dunite formation penetrated by numerous diabase sills. Those sills which can constitute 50% in volume of this new formation are often particularly thick, up to 7 m. They have been observed to branch to perpendicular dykes which probably represent their feeders. A few rodingitized gabbro dykes also intrude the ultramafic rocks but they are cut by the diabase ones. In contrast with the gabbros, the diabase dykes seem to be usually fresh or rodingitized only along their margins. As rodingitization probably accompanies the serpentinization of the peridotite[20], it can be concluded that the gabbro dykes were emplaced before the serpentinization and the diabase ones, after it or late during the process. The peridotite structure is not altered by the serpentinization except locally where low-temperature deformations have disrupted and boundinaged the dykes and developed a slickenside cleavage in the serpentinites. As a consequence the coarse porphyroclastic texture of the peridotite, typical of high-temperature flow[22] is still visible and its orientation can be mapped. The foliation is represented with its field orientation in Fig. 2 and with its restored orientation in Fig. 3. The thickness of

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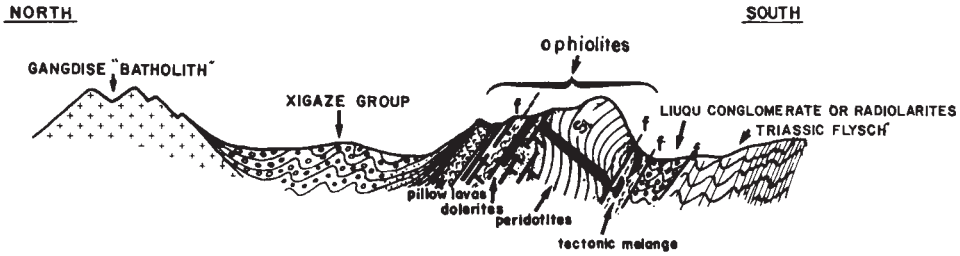

Fig. 2 Schematic north–south cross-section through the Yarlung Zangbo suture near Xigaze. S_1, foliation.

this harzburgite and dunite formation containing the sills is 500 m on average with important local variations. Downwards it grades into a 500-m unit formed of serpentinized foliated harzburgites and dunites with a decreasing amount of gabbro and diabase dykes. The degree of serpentinization rapidly decreases in these peridotites. After 1–2 km of relatively fresh harzburgites and dunites, fresh harzburgites with a crude layering resulting from the alternation of Cr diopside-rich and Cr diopside-poor facies, visible at various scales are encountered. No banding is observed in these rocks except for a few pyroxenites. The rocks are well foliated with discordant shear zones represented by a few metres of mylonitic peridotites in the Xigaze and Liuqu massifs (Figs 2, 3). Outside these bands, fine to coarse-grained porphyroclastic structures related to high-temperature plastic flow are observed. At the massif scale, the tectonic structures display a relatively irregular pattern though remaining consistent from one massif to another[17].

The southern east–west contact of the Xigaze ophiolite with the sedimentary formations is tectonic. Over 500–800 m, the ultramafic rocks are transformed into an ophiolitic mélange with blocks of rodingitized gabbros and diabases floating in a schistose serpentinite. On the other side of this major tectonic contact, either radiolarites or a coarse red conglomerate separate the ophiolites from the Triassic flysch series. They are also strongly deformed and schistozed at the contact but, like the serpentinites, are devoid of any metamorphism.

Amphibolites, garnet amphibolites and quartzites have been observed in several localities (Liuqu, Bainang and Dagzhuka) as inclusions in the serpentinite mélange. In the Dagzhuka massif, mylonitic peridotites are developed over several dozen metres above the basal contact which contains blocks of amphibolites in a serpentinite matrix. Beneath, a metamorphic formation consisting of quartzites, phyllites and locally ophicalcites, outcrops over 300 m. This might represent the basal part of the metamorphic aureole well known in many ophiolites[22,23].

Discussion

Considering the general tectonic situation, special attention must be devoted to the question of continuity within the ophiolites mainly at the critical level between the mafic and ultramafic formations. This continuity is thought to be locally preserved for the following reasons.

First, the sills and the dykes are remarkably continuous, keeping the same attitude from the volcanics down to the upper peridotites; and second, the same general sequence is systematically observed along the area studied (Fig. 1). In particular, the possibility of a squeezing out of the gabbros is remote, considering that no large masses of gabbros have been found. On the other hand, isolated pockets of gabbros are now well identified within the mafic part of the sequence. We thus think that the gabbros have not been faulted out and that a continuous section was observed in the Xigaze area.

The specific characters of the Xigaze ophiolite can be summarized as follows:

(1) In contrast with other non-dismembered ophiolites, the mafic part of the sequence is nearly devoid of plutonic rocks except for the small cumulate gabbro bodies found in the Dagzhuka and Tiding massifs. The mafic formation is entirely comprised of basaltic volcanics overlying diabases and dolerites with isotropic gabbro screens and, more rarely, trondjhemite screens.

(2) The diabase unit is a sill complex and not a dyke complex, as expected, with sills intrusive one into the other and only a few branching dykes at right angles to the sills. This has not yet been described in ophiolites except in the Point Sal ophiolite[24].

(3) This mafic part of the sequence is remarkably thin (compared with that measured in non-dismembered ophiolites) with local variations in thickness, but does not exceed 3 km.

(4) The upper harzburgites and dunites are invaded by thick diabase sills over a thickness of ~1 km. To our knowledge, this is also unique as diabase dykes are very scarce in the few ophiolite massifs where they have been described cross-cutting the harzburgites[25].

(5) Cr diopside-rich harzburgites are abundant in the ultramafic unit and appear only at 2 km beneath the mafic unit and at 5 km beneath the sedimentary cover of the ophiolite sequence. This is unique as the ultramafic unit in ophiolites is usually composed of depleted harzburgites with Cr_2 diopside-rich harzburgites appearing only in the basal section in a few massifs[27].

(6) Local thin shear zones inside the ultramafics with low-temperature plastic flow structures have not been described in ophiolites. Those observed are generally located at the base of the ultramafics. They are thicker and clearly related to late thrusting of the lithosphere[28].

Depending on the spreading rate, the structural, petrological and geochemical character of oceanic spreading centres are expected to vary. The difference seems to be essentially controlled by the heat budget[29,30]. Slow-spreading ridges will have a lower rate of magma generation and therefore of heat production for a comparable thermal dissipation rate. As a

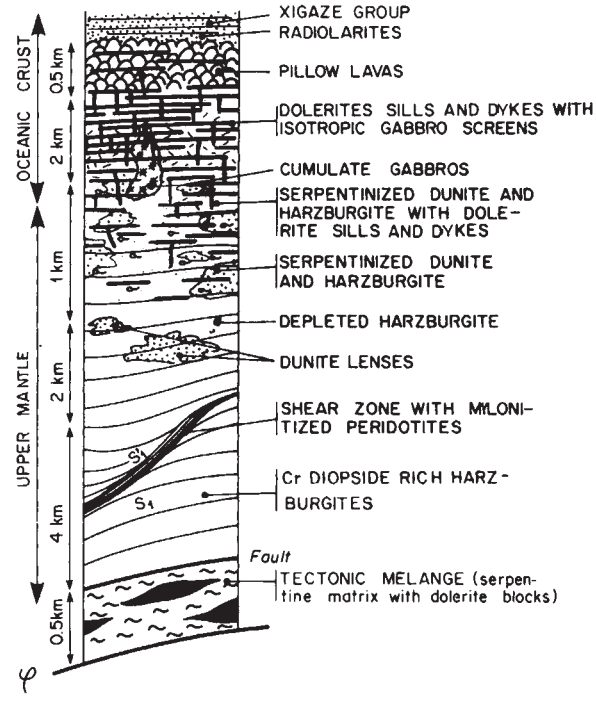

Fig. 3 Schematic cross-section of the ophiolite sequence. Solid lines represent the foliation S_1. The smaller the spacing, the higher the strain. The various attitudes have been restored after a rotation to horizontal of the radiolarites and basalt flows.

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result, magma chambers where the gabbro cumulate sequence crystallizes are not expected for spreading rates of <1 cm yr^{-1} (refs 31, 32). Steeper isotherms in the mantle should tend to concentrate the magmatic and tectonic activity closer to the ridge than in fast-spreading ridges. However, as mentioned elsewhere[33], one of the bases for these thermal models is that the upwelling rate of the asthenosphere is proportional to the rate of spreading. The fact that spreading is a discontinuous phenomenon is likely to change these conclusions.

If these conclusions are applied to the Xigaze ophiolite, our evidence indicates a crust formed at a spreading centre with a particularly slow spreading rate, <1 cm yr^{-1} if one accepts this limit for the transition from continuous to discontinuous magma chambers. This interpretation differs significantly from that of Wu Haoruo and Deng Wanning[14] who think—based on major element chemistry—that the Xigaze ophiolite has been formed in a subduction zone environment. Further detailed geochemical studies are necessary, however, as most of the studied mafic rocks are altered.

Although the mafic crust in our case is only ≤3 km on average, the geophysical Moho would be deeper, considering that the 1–2 km of serpentinized peridotites with up to 50% in volume of diabase sills display crustal seismic velocities. Our evidence that serpentinization occurred before the diabase emplacement shows that these peridotites were serpentinized at the ridge itself and therefore that they belong geophysically to the crust. The 'geophysical crust' would therefore have a thickness comparable with that determined by a V_p transition from 6.8 km s^{-1} to 8.1 km s^{-1} for slow ridges like the Atlantic one[34].

Finally, if the interpretation that the Xigaze ophiolite originated at a slow ridge is correct, the study of its structure, petrology and geochemistry bears directly on the structure and nature of slow ridges. In this respect, the occurrence of a sill complex as the main component of the crust, which also extends in the uppermost mantle, is interesting.

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Antibodies to left-handed Z-DNA bind to interband regions of *Drosophila* polytene chromosomes

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Antibodies which are specific to the Z–DNA conformation have been purified and characterized on the basis of their binding to three different DNA polymers which can form this left-handed helix. These antibodies bind specifically to polytene chromosomes of Drosophila melanogaster *as visualized by fluorescent staining. The staining is found in the interband regions and its intensity varies among different interbands in a reproducible manner. This is the first identification of the Z-DNA conformation in material of biological origin.*

LEFT-HANDED double-stranded DNA was first discovered by an atomic resolution X-ray crystallographic analysis of the hexanucleoside pentaphosphate CpGpCpGpCpG (ref. 1). In this conformation the DNA formed a double helix with Watson–Crick base pairs and antiparallel sugar–phosphate chains; the sugar–phosphate backbone followed a zig-zag course and for this reason was named Z-DNA. In Z-DNA the guanine bases have rotated about the glycosydic bond and have assumed a *syn* conformation, in contrast to the *anti* conformation in B-DNA. The early observation by Pohl and Jovin[2] of salt-induced inversion of the circular dichroism spectrum of poly(dG-dC) DNA in 4 M NaCl solution can now be understood as the conformational transition of the right-handed B-DNA to the left-handed Z-DNA[3,4]. Z-DNA has been seen in several crystal structure and fibre analyses[5–8] and generally, DNA with alternating purine–pyrimidine sequences may be expected to have Z-forming potential.

We have recently demonstrated that left-handed Z-DNA is a strong immunogen; in both rabbits and mice antibodies can be raised which are specific for the left-handed Z conformation and will not react with right-handed B-DNA[9]. Because of their specificity, antibodies can be used to determine the distribution

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of Z-DNA in biological systems. Here we have purified antibodies to Z-DNA from rabbit sera and demonstrate their specificity for the left-handed Z conformation. We have used these antibodies for indirect immunofluorescent staining of *Drosophila melanogaster* polytene chromosomes. Because a large polytene chromosome can consist of a thousand or more individual chromatids in very precise alignment, these chromosomes provide a magnification that cannot be obtained with other types of chromosomes. In addition, polytene chromosomes are interphase chromosomes, active in both transcription and replication, in spite of the relatively condensed state of the chromatin. The alignment of the many chromatids making up a polytene chromosome is so precise that regions of tight coiling in the individual chromatids match up to give the appearance of bands across the width of each chromosome. These bands are separated by interbands in which the chromatid fibres are relatively extended. The banding pattern of each chromosome is essentially constant from nucleus to nucleus and from individual to individual, and must thus reflect basic features of chromatid structure, although the significance of this structure is not understood. Polytene chromosomes therefore provide a unique opportunity for studying the conformational states of specific chromatin regions at times when the chromatin is genetically active.

Indirect immunofluorescence has been widely used to study the distribution of proteins on polytene chromosomes[10], and here we apply this technique to investigate the occurrence and distribution of Z-DNA in these chromosomes. We show that anti-Z-DNA antibodies bind to polytene chromosomes in a very reproducible pattern, exhibiting fluorescence staining exclusively in interband regions in our experimental conditions. This finding may throw some light on the role of Z-DNA in biological systems and may lead to some clues concerning the significance of banding in eukaryotic chromosomes.

Characterization of the antibody

As described earlier[9], the anti-Z-DNA antibodies were raised by injecting rabbits with brominated poly(dG-dC)·poly(dG-dC). This DNA polymer remains in the Z-form even in low salt because most of the bromine atoms are on the C-8 position of guanine, stabilizing guanine in the *syn* conformation. In low salt, non-brominated poly(dG-dC)·poly(dG-dC) is in the B conformation and does not react with the antibody. In 4 M NaCl the unmodified poly(dG-dC)·poly(dG-dC) is converted to the Z form and does react with the anti-Z-DNA antibody.

Although our initial experiments on polytene chromosomes were carried out with whole sera from rabbits injected with brominated poly(dG-dC)·poly(dG-dC), the more recent experiments have been done with antibodies purified from these sera. The purified antibody was obtained by preparative scale quantitative immunoprecipitation in 4 M NaCl using unmodified poly(dG-dC)·poly(dG-dC), which is in the Z conformation at that salt concentration[2,4]. The specificity of the affinity-purified antibody for the Z conformation was tested on native *Escherichia coli* DNA and three different polymers, poly(dG-dC)·poly(dG-dC), brominated poly(dG-dC)·poly(dG-dC) and the methylated polymer poly(dG-m^{5}dC)·

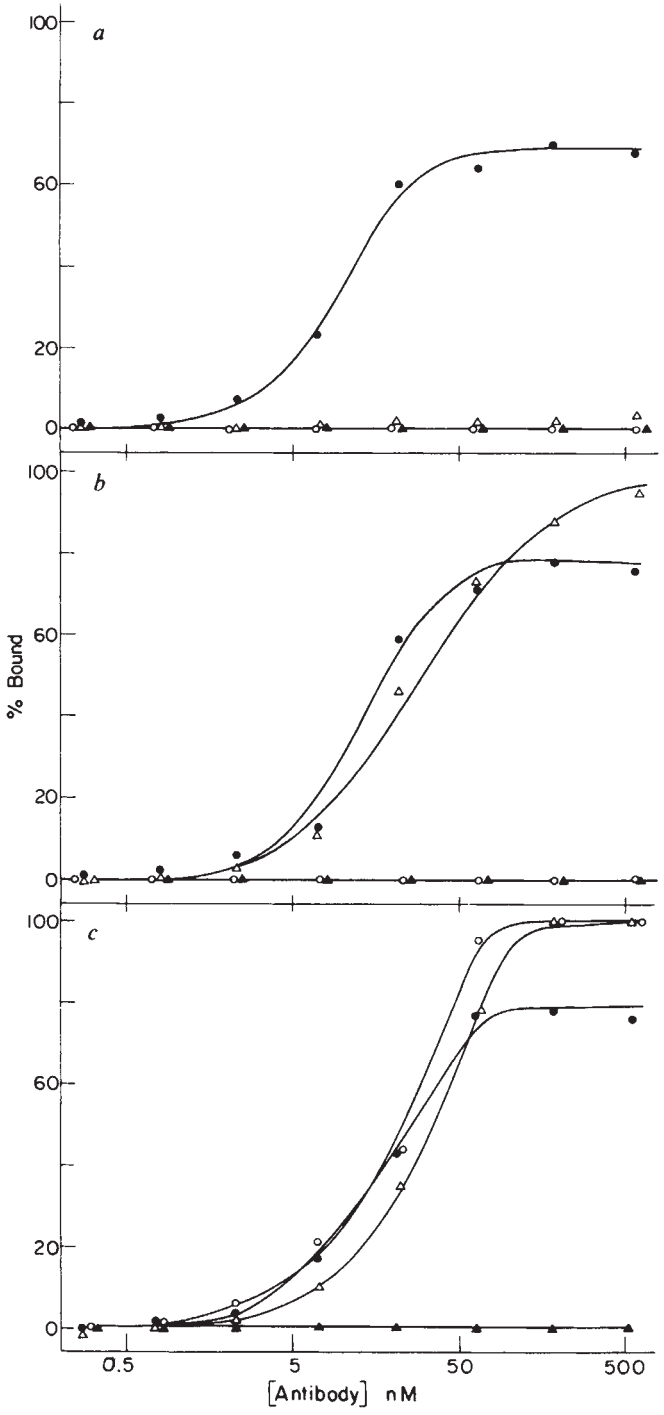

Fig. 1 Specificity of the affinity-purified anti-Z-DNA antibody as measured by direct binding to ^{3}H-brominated poly(dG-dC)·poly(dG-dC) (●), ^{3}H-poly(dG-m^{5}dC)·poly(dG-m^{5}dC) (△), ^{3}H-poly(dG-dC)·poly(dG-dC) (○) and ^{3}H-labelled *E. coli* DNA (▲) in 0.2 M NaCl (*a*), 1.5 M NaCl (*b*) and 4.0 M NaCl (*c*). The affinity-purified antibody was prepared by a modification of the procedure of Kitagawa and Okuhara[22]. A sample of rabbit serum was incubated with an equivalent amount of poly(dG-dC)·poly(dG-dC) (as determined from quantitative precipitation curves) in 60 mM sodium phosphate, 30 mM EDTA, 4 M sodium chloride, *p*H 8.0, for 3 h at 37 °C and overnight at 4 °C. For equivalent proportions of antigen and antibody, 75 A_{260} units of polynucleotide were added to 15 ml of serum. Resulting precipitates were washed three times with cold PBS (10 mM sodium phosphate, 140 mM sodium chloride, *p*H 7.4) and dissolved in 20 mM sodium carbonate, 5% dimethyl sulphoxide (DMSO) *p*H 10.5. The dissolved antibodies and antigen were separated on a DEAE-cellulose column (20 ml) previously equilibrated with 20 mM sodium carbonate, 5% DMSO, *p*H 10.5. The free antibody flowed through on washing with 20 mM sodium carbonate, 5% DMSO, *p*H 10.5. The retained nucleic acid was eluted by 20 mM sodium carbonate, 5% DMSO, 1 M NaCl, *p*H 10.5. The purified antibodies were dialysed against PBS and protein concentration determined by the method of Lowry *et al.*[23]. Radioimmunoassays (RIAs) were performed by incubating 100 μl samples of purified antibody serially diluted into the RIA buffer (60 mM sodium phosphate, 30 mM sodium EDTA, *p*H 8.0) containing 0.2 M NaCl (*a*), 1.5 M NaCl (*b*) or 4.0 M NaCl (*c*) with 100 ng of ^{3}H-labelled nucleic acid in 0.05 ml of the RIA buffer containing the indicated NaCl concentration for 1 h at room temperature. Then 0.05 ml of the γ-globulin fraction of goat anti-rabbit immunoglobulin serum in PBS was added and the mixture kept at room temperature for an additional hour. The resulting precipitate was centrifuged, washed twice with RIA buffer containing the indicated NaCl concentration, dissolved in 1 ml 0.1 N NaOH and counted in 10 ml of Aquasol-2 (NEN). ^{3}H-thymidine-labelled *E. coli* DNA was prepared from a mutant strain B3, as described previously[24], but with a pulse of 0.5 mCi of ^{3}H-thymidine. ^{3}H-labelled poly(dG-m^{5}dC)·poly(dG-m^{5}dC) was synthesized according to Behe and Felsenfeld[11], except that 0.065 mCi of ^{3}H-dGTP (Amersham) was included into the synthesis reaction mixture. The other polymers were radiolabelled by nick translation[25].

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poly(dG-m^{5}dC). Behe and Felsenfeld have shown that methylation on the 5 position of cytosine yields a polymer that is converted to Z-DNA at low concentrations of $MgCl_2$, and has a midpoint for conversion by NaCl at 700 mM (ref. 11).

Figure 1 demonstrates the specificity of the purified antibody by showing its direct binding as a function of antibody concentration in three different salt environments using the four polymers. In 0.2 M NaCl, the antibody bound only the brominated poly(dG-dC)·poly(dG-dC) (Fig. 1*a*), the only one which has the Z conformation in 0.2 M NaCl. At 1.5 M NaCl, antibody bound the brominated and methylated polymers, both of which have the Z conformation, but not the two polymers which are in the B conformation at that ionic strength (Fig. 1*b*). In 4 M NaCl the brominated, methylated and unmodified poly(dG-dC)·poly(dG-dC) were bound, but *E. coli* DNA was not (Fig. 1*c*). Poly(dG-dC)·poly(dG-dC) is completely in the Z-DNA conformation in 4 M NaCl, where its Raman spectrum is identical with that of Z-DNA crystals[4]. The three experiments shown in Fig. 1 strikingly illustrate the effect of antigen conformation on the reactivity of the antibody. Only when the polymers are in the Z conformation does the affinity-purified antibody combine with the DNA antigen. When the antigen is not in the Z conformation, no reaction occurs. The same results were obtained with whole serum.

Further analyses on the specificity of the antibody were done by competitive radioimmunoassays in which the ability of other polymers to inhibit the binding of the antibody to a known form of Z-DNA was measured. No inhibition was seen with poly(dG), brominated poly(dG), poly(dG)·poly(dC), brominated poly(dG)·poly(dC), native and denatured calf thymus DNA, Vero RNA, DNA–RNA hybrids or double-stranded RNAs. All our experiments indicate that the antibody is highly specific for Z-DNA and does not recognize B-DNA or other nucleic acid conformations.

Binding of antibodies to polytene chromosomes

Identical results were obtained when either the antibodies in whole serum or the affinity-purified antibodies were used to look for the presence of Z-DNA in polytene chromosomes from larval salivary glands of *D. melanogaster*. Antibodies bound to the chromosome are detected by the binding of a second antibody, a fluorescein-labelled goat antibody to the rabbit immunoglobulin. The second antibody also provides an amplification of the binding of the primary antibody, as several molecules of the goat antibody can bind to each rabbit antibody.

The anti-Z-DNA antibody stains the polytene chromosomes in a very distinct and specific way (Fig. 2*a*). It can be seen that there are many fluorescent regions on all the chromosome arms as well as a somewhat diminished and diffused staining in the chromocentre, where the arms are joined. All the control experiments described above have indicated that the antibody is recognizing the Z conformation. Other control experiments indicate that the antibody binding to the polytene chromosomes is also the antibody that recognizes the Z conformation (Fig. 2*b*,*c*). For the preparation shown in Fig. 2*b* the antibody was incubated with B-DNA, in the form of poly(dG-dC)·poly(dG-dC), before it was put on the chromosome preparation. The antibody staining of the chromosomes is not affected. However, when the antibody was preincubated with Z-DNA in the form of brominated poly(dG-dC)·poly(dG-dC), the chromosomal staining is completely abolished (Fig. 2*c*), and chromosomes appear uniformly dark. Note that preparations stained with antibody preincubated with Z-DNA reproducibly show more background fluorescence than do other preparations. This is because the antigen–antibody complex has precipitated throughout the field and around the outside of the chromosomes. This complex has not been washed away and has thus reacted with the fluorescent goat anti-rabbit antibody. Experiments in which the antibody was preincubated with either native or denatured DNA from *Drosophila* embryos showed no effect on chromosomal staining.

The same chromosomal staining pattern is produced by whole serum from rabbits injected with brominated poly(dG-dC)·poly(dG-dC), by antibodies affinity purified from this serum by the brominated polymer in low salt, or by antibodies affinity purified from the serum by unmodified poly(dG-dC)·poly(dG-dC) in high salt where this polymer is in the Z conformation. We have also studied an antibody obtained from a rabbit injected with unmodified poly(dG-dC) poly(dG-dC), which exists as B-DNA in physiological salt solution. The antibody produced by this rabbit was of lower titre but was specific for Z-DNA at the dilution used in the experiment. To obtain this antibody, the polymer was injected into the rabbit complexed with methylated bovine serum albumin and it is assumed that the cationic charges on the surface of the methylated bovine serum albumin provided regions which converted local domains of the poly(dG-dC)·poly(dG-dC) into Z-DNA[9]. This last antibody produced without bromine atoms yielded the same chromosomal staining pattern as the other antibody

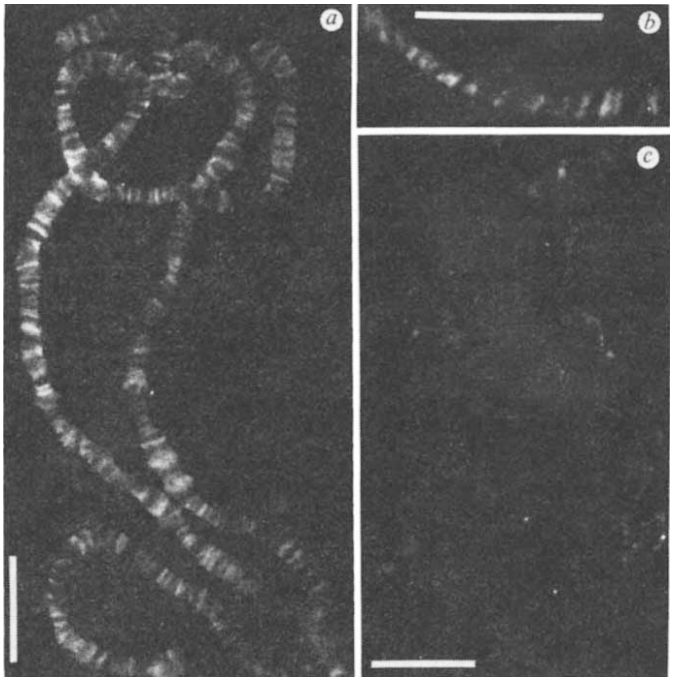

Fig. 2 Fluorescence micrographs of polytene chromosomes demonstrating the specificity of the reaction between anti-Z-DNA antibody and fixed chromosomes. *a*, Chromosomes stained with anti-Z-DNA antibody by our standard procedure. *b*, Chromosomes stained with anti-Z-DNA antibody, which had been preincubated with poly(dG-dC)·poly(dG-dC) in the B conformation. The pattern of fluorescence staining is identical to that produced by the antibody without B-DNA competitor. *c*, Chromosomes stained with anti-Z-DNA antibody, which had been preincubated with the Z-form brominated poly(dG-dC)·poly(dG-dC). No chromosomal fluorescence staining is detectable although the precipitated antigen–antibody complex produces an increased general background of fluorescence. Salivary glands were dissected out of third instar Oregon R larvae (gt-1 stock) into a droplet of 45% acetic acid in Ringer's buffer on a coverslip. After 2–5 min a microscope slide was touched to the drop on the coverslip, the gland was squashed mechanically and the slides were placed on the flat surface of a block of dry ice. After 20 min freezing on dry ice the coverslips were pried off and the slides were briefly immersed horizontally in PBS. Excess PBS was removed from the slide without completely drying the surface; 0.02 ml antibody-containing PBS solution was then added and incubated for 15 min at 37 °C. The slides were then gently washed in PBS and fluorescein isothiocyanate (FITC) conjugated IgG fraction of goat anti-rabbit serum (Cappell) was added at a 1:250 dilution in PBS, followed by another 37 °C incubation for 15 min. Unbound FITC-conjugated secondary antibody was gently washed out and a clean coverslip was mounted with a drop of PBS. The slides were viewed and photographed in a Zeiss microscope using both incident UV illumination and phase contrast optics. Photographs were taken using Kodak Ektachrome 400 film. For the blocking experiments of *b* and *c*, the antibody solutions contained the respective competitor DNAs at concentrations of 0.5 mg ml^{-1} and were preincubated for 30–45 min at 37 °C before staining of the polytene chromosomes. Scale bar, 10 μm.

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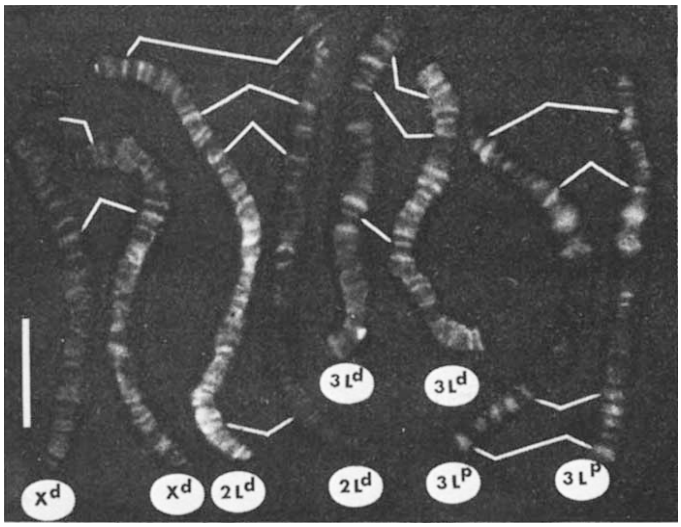

Fig. 3 Fluorescence micrographs of chromosomes stained with the anti-Z-DNA antibody demonstrating the constancy of the fluorescence pattern from nucleus to nucleus. Sections of chromosomes from different nuclei are aligned and selected fluorescent regions are marked to orient the viewer. d marks the most distal sections of chromosome arms X, 2L and 3L; p designates the proximal part. Fluorescence staining and visualization was done as described in Fig. 2. Scale bar, 10 μm.

preparations. None of the normal rabbit sera which we have used nor incubation with fluorescent labelled goat anti-rabbit antibody alone has produced staining of the polytene chromosomes. Thus, all our control experiments indicate that the antibody is recognizing the Z-conformation in the polytene chromosomes, as it does in the immunoprecipitation experiments.

The anti-Z-DNA antibodies produce a distinctive pattern of staining on the polytene chromosomes; the fluorescent segments seen on the chromosome vary both in size and intensity, but the size and staining intensity of any particular region is reproducible and appears to reflect the nature of the chromatin. Figure 3 shows several examples of chromosomal segments from different nuclei. The reproducibility of the staining pattern is most conveniently seen when the two homologues that make up a chromosome have become unpaired along a part of their length. Figure 4 shows two examples that demonstrate the similarity of the staining pattern in the asynapsed chromosomes.

Some of the fluorescence photographs in Figs 2–4 were exposed for long periods to give a stronger fluorescent signal. However, photographs were also taken with a shorter exposure so that the image would be in the linear range of the film response, and here, the darker non-fluorescent segments were as dark as the general background. From analyses of negatives of these photographs using a densitometer tracing to look at the variations in the amplitude, we estimate that the intensity of different fluorescent segments roughly varies by a factor of 5–10 in brightness.

To determine which segments of the *Drosophila* chromosome are fluorescing, we compared the pattern of bands seen in phase microscopic photographs with the fluorescence pattern (Fig. 5). Photographs of segments from various parts of the *Drosophila* chromosome were cut along the chromosomal axis and the phase photographs were compared with the fluorescence photographs. Figure 5 shows that the fluorescent segments are the interbands and not the bands. The bright areas in the fluorescence photograph are found in the position of the light interband regions in the phase photographs. Note that there is not necessarily a correlation between the width of the interband segment and its fluorescent intensity.

Thus, the antibody binding results shown in Fig. 1, together with the negative controls, clearly demonstrate that we are using an antibody that is specific for the Z-DNA helix. It reacted with three polymers only in distinct conditions required for each to occur in the Z conformation, and did not react with *E. coli* DNA in any of these conditions, nor with single-stranded DNA and a variety of polynucleotides, including brominated polymers. The bromination was not required either for induction of Z-specific antibody or for its serological reactivity. The antibody that binds to polytene chromosomes shows the same properties as those measured in the binding experiments; chromosomal staining is blocked only by molecules that have a Z conformation and yield an immune precipitate with the antibody.

Tests for effects of experimental procedures

As the chromosomes in almost all cytological preparations have been subjected to treatments that remove or alter cellular components, this raises the question of whether sample preparation induces formation of Z-DNA in the polytene chromosomes. At present, the only way we can approach this question is by testing the effects of various preparative procedures, and we have therefore tested preparations from salivary glands that were incubated for 2–5 min at room temperature in 45% acetic acid, squashed in the same solution, frozen on dry ice and treated in various ways before incubation with antibody. After freezing and removal of the cover slips, the slides were: (1) placed directly in phosphate-buffered saline (PBS), or (2) immersed in ethanol, air dried and then placed in PBS, or (3) placed directly in a post-fixative solution containing 3.7% formaldehyde and then in PBS. Alternatively, glands were fixed by the formaldehyde technique of Silver and Elgin[12], squashed in 45% acetic acid with 10 mM $MgCl_2$ and frozen on dry ice, then immersed in ethanol and air dried before incubation with antibody in PBS.

These treatments might be expected to produce different types of artefact, yet all result in qualitatively the same staining pattern. The first procedure produces by far the most intense staining pattern and has been used as our standard preparative technique. Less intense staining occurred when formaldehyde was used for fixation and so further study will be required to determine whether bright staining was due to selective removal of proteins during exposure to 45% acetic acid. In the post-fixed preparations it is likely that the formaldehyde is itself blocking the antibody binding site.

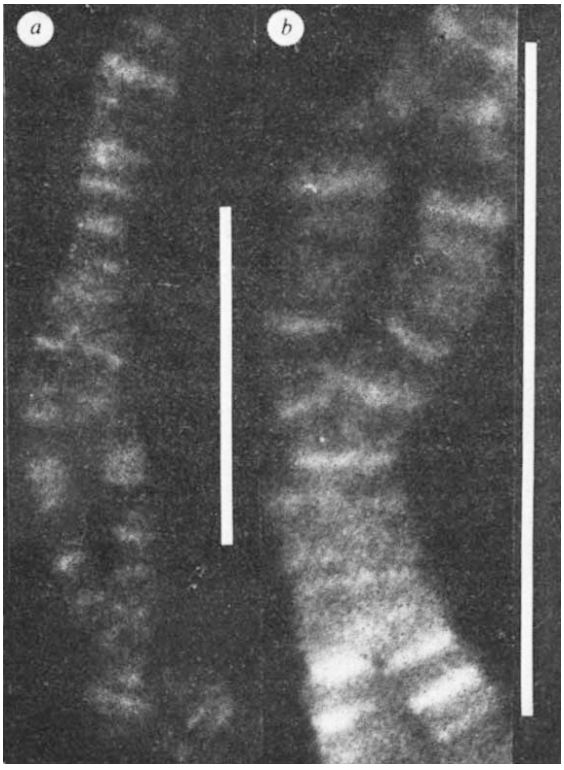

Fig. 4 Reproducibility of the anti-Z-DNA antibody staining pattern as seen in two examples of asynapsis (*a*, *b*). The unpaired homologues of a polytene chromosomal arm show very similar fluorescence staining in the homologous regions. Experimental protocol was as described in Fig. 2.

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The polytene chromosome is segmented into bands and interbands. The bands vary in size, yielding a banding pattern that is invariant unless chromosomal rearrangements occur. These banding patterns are useful for genetic mapping studies and for studies of evolutionary relationships between species; however, their role in chromosome structure is unknown. Banded polytene chromosomes have also been reported in several other eukaryotic organisms including protozoa and plants[13]. It has been suggested that this banding might be a general property of the organization of the eukaryotic chromosome[13]. *Drosophila* has ~5,000 bands as well as interbands which have been identified and mapped. On average, a band plus interband contains some 30 kilobases of DNA[13]. The DNA in the interband has been estimated as ranging from 5% (ref. 13) to 30% (ref. 14) of the total DNA, depending on the method of measurement. Electron micrographs of *Drosophila* chromosomes show bands as containing darkly staining, densely clumped units while the interbands are visualized as elongated fibrils 50–60 Å in diameter[13]. Indirect immunofluorescence has revealed a number of proteins in the bands[15–17] and interbands[16–18].

An important question in any immunofluorescence experiment is whether the antigen is present but not accessible for reaction. This question is especially relevant to our experiments because staining is limited to the least compact regions of the chromosomes. Although anti-histone antibodies can cause brighter staining in the more compact regions than in interbands[15] it is possible that the DNA, or a particular portion of it, is not as available as the histones. Although we cannot rule out the presence of Z-DNA in the band regions, our experiments do show that it is either more abundant or more accessible in the interbands. Either of the alternatives is interesting and a decision between the alternatives may help explain the significance of chromosomal banding. This question is under further study with antibodies that react with other forms of DNA.

The reproducibility and uniformity of the staining pattern throughout the entire length of the polytene chromosome are remarkable. We have compared the staining pattern among different giant polytene chromosomes within an individual and between individuals. The staining pattern of Z-DNA antibodies appears to be a constant feature of the chromosomes in much the same way that the banding in the phase contrast microscope appears constant. This suggests that the pattern is an intrinsic property of the chromosome rather than, for example, a property induced through the mechanical action of squashing the chromosome, for then one would expect significant differences in the staining pattern in various parts of the chromosome, depending on the degree of mechanical stress to which each segment was subjected.

The intensity of staining in different interbands varies considerably, and seems to be unrelated to the width of the interband; the origin of the variability is not known. The possibility that the brighter segments contain a larger number of closely packed and unresolved band–interband units is unlikely, because we have occasionally seen extremely stretched regions in which the interbands are considerably extended and still do not break up into smaller fluorescent segments.

It would be interesting to know the proportion of the interband DNA that needs to be Z-DNA to produce the observed fluorescent pattern. This is difficult to estimate because we have no absolute standard which will allow us to determine accurately the amount of primary antibody bound from the amount of fluorescence detected. As a lower limit it is possible that only a small stretch of some 6–8 base pairs out of the 3 kilobase pairs in the average interband might be in the Z conformation. In a polytene chromosome, where 1–2,000 copies of such a sequence are lying in register, one would have enormous numbers of antibodies arrayed transversely across the interband. Because of the limited optical resolution in the light microscope and the broadening of the fluorescent image, one site would appear to occupy the entire 0.1 μm of an average interband. As an upper limit, the entire interband could be in the Z-DNA conformation. Although this seems implausible, further work will be required before we can make a more accurate estimate.

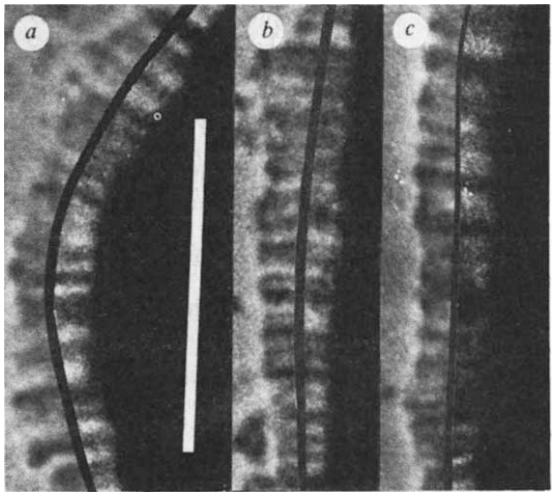

Fig. 5 Comparison of phase contrast and anti-Z-DNA antibody-stained fluorescent photographs of polytene chromosomes. Chromosome sections from three different nuclei (*a–c*) are shown. Analogous sections of the same chromosome are compared by cutting the photographs down the chromosomal axis and comparing the two halves. The light areas of the left phase contrast photographs correspond to the interbands while the darker sections are the bands. The white sections of the right darkfield fluorescent photographs show the position of the anti-Z-DNA fluorescent stained antibodies. It can be seen that the interbands are fluorescing. Note that the intensity of fluorescence is not necessarily related to the width of the interband. Fluorescence staining was done as described in Fig. 2 legend. Scale bar, 5 μm.

Possible functional significance of Z-DNA

Because the Z conformation is a reversible structural form of DNA it is an attractive candidate for having a regulatory role in genetic activity. Many regions of the polytene chromosomes undergo changes in chromatin structure, called puffs, which are associated with transcription of certain genes. The patterns of Z-DNA staining during such structural changes will be of obvious interest.

In physiological salt conditions the Z-DNA conformation is somewhat less stable than the B conformation and therefore has to be stabilized. There may be four ways of maintaining the Z conformation in biological systems. (1) Supercoiling. Z-DNA twists the double helix in a left-handed mode, opposite to the right-handed B-DNA conformation. Because of this one can interchange strongly negative supercoiled DNA for segments of Z-DNA (L. Peck, A. N., A. R. and J. C. Wang, in preparation). (2) Binding to proteins which are specific for the Z conformation. These probably involve electrostatic interactions with basic residues. (3) Binding to specific ions. For example, spermine or spermidine can stabilize crystals of Z-DNA so that they form with a regularity which yields an atomic resolution diffraction pattern[1,7]. (4) Modification such as methylation of cytosine in the 5 position (although not necessarily applicable to *Drosophila* DNA[19]). In the absence of methylation poly(dG-dC)·poly(dG-dC) requires 700 mM Mg^{2+} to convert to the Z conformation. Behe and Felsenfeld[11] have shown that when the methyl group is present, the Z conformation is formed in 0.6 mM Mg^{2+}. It is interesting that spermine is even more effective, forming the Z conformation at a concentration of 0.002 mM. Further work must be done before we fully understand the parameters which stabilize the Z conformation in the polytene chromosome.

Work with synthetic deoxypolynucleotides has amply demonstrated that there is a reversible equilibrium between the right-handed B conformation and the left-handed Z conformation in molecules in which there is an appropriate sequence. Recent work with cloned segments of poly(dG-dC)·poly(dG-dC) in plasmids have demonstrated that it is possible for these segments to undergo a B to Z transformation even though they

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are enclosed in plasmid segments containing B-DNA (ref. 20 and L. Peck, A. N., A. R. and J. C. Wang, in preparation). It is thus reasonable to imagine that appropriate segments in chromatin might undergo similar changes in conformation. Z-DNA segments may have the property of not only changing the local environment near a particular gene, but through interaction with supercoiling these segments could modify the transcribability of DNA regions far removed from the site at which the Z-DNA segment is found.

The striking observation here is that an antibody specific to left-handed Z-DNA binds in a regular fashion to the interband regions of the *D. melanogaster* polytene chromosomes. Our control experiments strengthen the conclusion that this binding is due to a Z-DNA conformation found in the interbands. Band–interband units seem to be basic units of gene activity[13] replication[21] and perhaps chromosomal synapsis. It is tempting to suppose that Z-DNA might be a conformational switch which is involved in the control of transcription or of other of these activities. As Z-DNA appears to be a fundamental component of the polytene chromosome, these chromosomes may provide a unique opportunity for further exploration of the role of Z-DNA.

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Dual function transcripts specifying tRNA and mRNA

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A cluster of four tRNA genes in Escherichia coli *is co-transcribed with an adjacent gene encoding elongation factor Tu. The resultant transcript that specifies both structural (tRNA) and informational (mRNA) RNA may not be an uncommon occurrence and has interesting regulatory implications.*

In *Escherichia coli*, where regulation of transcription has been most extensively studied, the RNAs synthesized are generally divided into two categories—the class of informational or mRNAs that are metabolically unstable, and the class of structural RNAs such as rRNA and tRNA that do not show appreciable turnover. The two classes are also distinguishable by their regulation pathways. In particular, stable RNA is subject to a coordinate regulation that is responsive to the availability of amino acids for protein synthesis. In general, this type of control only affects informational RNAs encoding proteins required in protein synthesis.

Transfer RNA genes are distributed throughout the *E. coli* genome as part of ribosomal RNA operons or as clusters of tRNA genes containing either different tRNA genes or repeats of the same gene (for review see ref. 1). We report here a novel transcription unit composed of a tRNA cluster and mRNA and suggest that the co-transcription of structural and informational RNA may not be an uncommon occurrence. Possible implications of these dual function transcripts are also discussed.

Organization and structure of the *tyrU* gene cluster

The *tyrU* gene cluster is situated between the *rrnB* ribosomal operon and the *tufB* gene at 89 min (ref. 2). The *tyrU* locus encodes the major tyrosine accepting species, $tRNA_2^{Tyr}$, which occurs as a single copy within a cluster of three other tRNA genes[3–5]. The *tufB* locus codes for elongation factor (EF)-Tu, which promotes the binding of aminoacyl-tRNA to ribosomes. In addition to its key role in translation, EF-Tu functions as a structural component of Qβ replicase (see ref. 6 for review).

Figure 1 shows the DNA sequence of the *tyrU* gene cluster and the 5′ end of the adjacent *tufB* gene, which is located only 114 base pairs (bp) downstream of the tRNA cluster. The *tyrU* promoter (as identified by hybridization analyses presented below) is located directly upstream of the first tRNA gene in the cluster and shares two features with the *tyrT* promoter region: a region of G-C bias flanking the Pribnow box (Fig. 1 and ref. 7), and wo dyad symmetries, one involving the −35 region and the other centred near the Pribnow sequence (Fig. 1 and ref. 8). These sequence features are present in the promoter regions of other stringently controlled genes and may therefore have a role in their coordinate synthesis. The Pribnow box participates in a dyad symmetry in the ribosomal protein operons[9] and the G+C-rich sequence following the Pribnow heptamer has been noted for rRNA and ribosomal protein operons[10].

A co-transcript of tRNA and mRNA

Knowledge of the organization and structure of the *tyrU* cluster and adjacent *tufB* gene has facilitated analyses of the *in vivo* transcripts and processing intermediates. The methods developed by Alwine and co-workers[11] were used to detect RNA species that comprise a small fraction of the total cellular RNA. The experimental approach involved fractionating denatured

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RNA by gel electrophoresis, transferring the RNA to diazobenzyloxymethyl (DBM) paper and probing the RNA blots with radiolabelled restriction fragments isolated from the *tyrU* region (as detailed in Fig. 2 legend). Detection of RNA precursors was maximized by using *E. coli* strains deficient in RNA processing. Precursor tRNAs accumulate in these strains as a result of mutations blocking and/or delaying their processing[12].

Figure 2 shows results of this analysis for selected probes. The tRNA-specific probe (Fig. 2 *B*) hybridized to one band of 1,800 bases (transcripts smaller than ~700 bases were detected on the higher composition gels shown in Fig. 5). The *tufB* probe (Fig. 2 *C*) also hybridized to the 1,800-base transcript, as well as to four other transcripts sized at 4,700, 1,550, 1,420 and 1,290 bases. As detailed below, the 4,700-base transcript contained sequences from the other gene encoding EF-Tu, *tufA* (ref. 13), and the lower molecular weight transcripts probably represent *tufA* and *tufB* processing intermediates. The probes immediately upstream (Fig. 2 *A*) or downstream (Fig. 2 *D*) of the tRNA-*tufB* region did not hybridize to the 1,800-base transcript. This collection of probes thus defines a transcript of 1,800 bases which contains sequences from both the tRNA gene cluster and the *tufB* gene.

The 1,800-base transcript was further characterized by sequential hybridizations using probes covering different portions of the *tyrU* region. Figure 3 summarizes the hybridization results. Eight probes isolated from regions of structural tRNA or *tufB* sequence displayed hybridization to the 1,800-base transcript. Importantly, the sum of the restriction fragments that hybridized to the co-transcript corresponds to the molecular weight of the transcript (1,800 bases) determined on denaturing gels. Probes that failed to hybridize to the 1,800-base transcript defined the outer limits of the transcript: the 5′-proximal probe that produced negative results was separated from the structural $tRNA_4^{Thr}$ sequence by 12 bp and the 3′-proximal probe began 85 bp downstream of *tufB* structural sequence. Thus, this series of probes specified a transcript encompassing both the tRNA cluster and the *tufB* gene.

To establish these unexpected results unequivocally, control experiments were carried out to ensure that: (1) failure to detect hybridization with the negative probes was not due to insufficient radioactivity, and (2) the observed hybridization with the positive probes was not the result of either contamination (with other labelled DNA) or the result of fortuitous homologies. The ability of each nick-translated probe to produce a strong hybridization signal to its respective band and to no other fragments of a digest of λ rif[d]18 DNA was tested. As

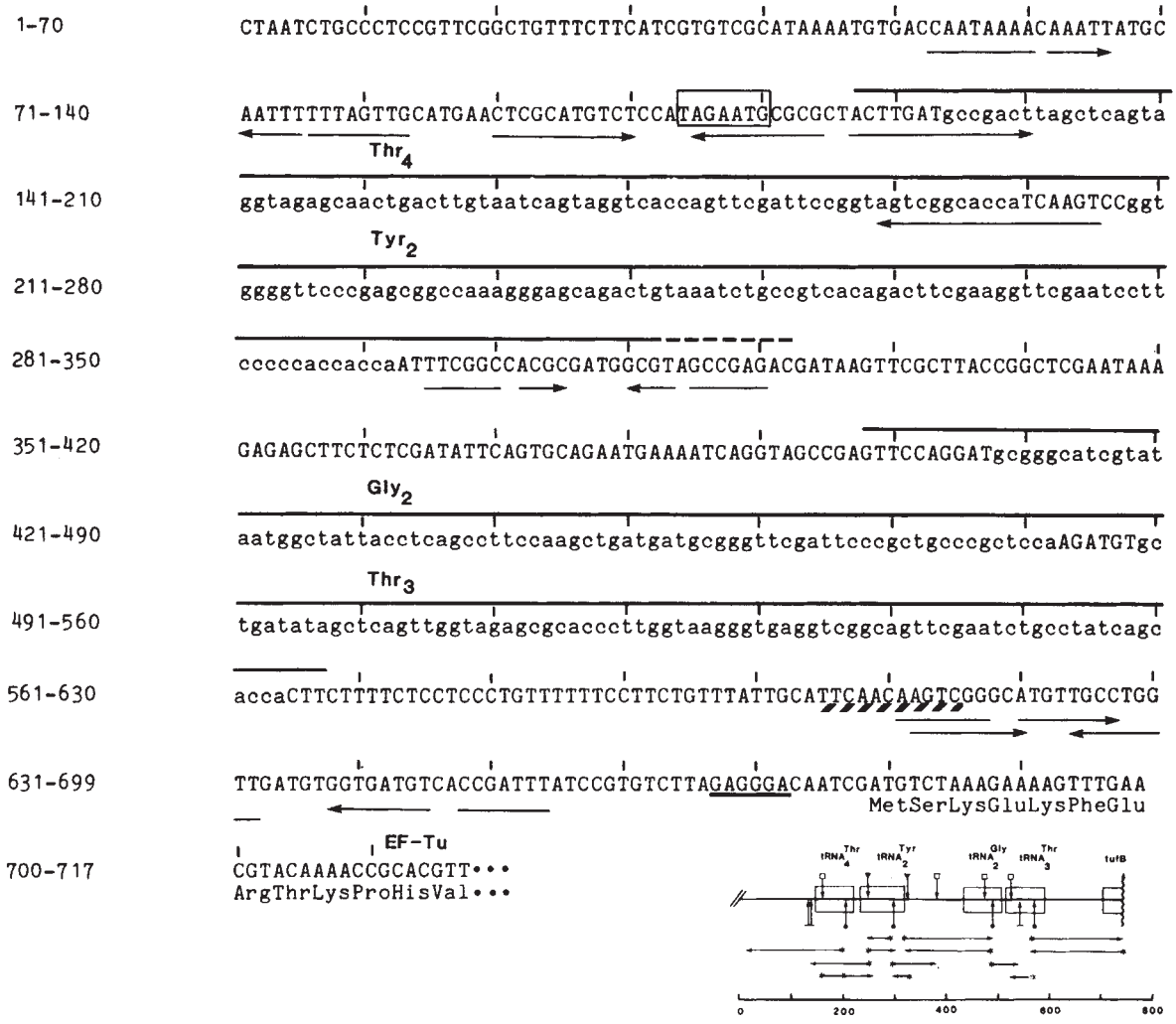

Fig. 1 Nucleotide sequences of the *tyrU* cluster and proximal *tufB* region. The DNA strand with the sequence of the RNA transcript is shown. The source of DNA was the transducing phage λ rif[d]18 (ref. 51; supplied by M. Nomura or N. Fiil). DNA sequencing was by the method of Maxam and Gilbert[52]; *, 5′-labelled end of the restriction fragments; →, direction and extent of the sequence obtained; the scale is in base pairs. Complement and overlap were obtained for all new sequences reported here with the exception of the sequence upstream of the $tRNA_4^{Thr}$ gene. The sequence is identical to that reported by An andFriesen[16] with the exception of an additional base pair they place at position 317. Structural tRNA sequence (lower case) and the two dimeric precursors are overlined. The $tRNA_2^{Gly}$–$tRNA_3^{Thr}$ dimer is 170 bases. The precise 3′ terminus (– – –) of $tRNA_4^{Thr}$–$tRNa_2^{Tyr}$ (~200 bases) is not known. It is located in a region of dyad symmetry (→ ←) as is the site between the tRNA cluster and the *tufB* gene at which processing or termination occurs (positions 608–618; see text). Transcription from the presumptive promoter would initiate at the A 7 bases upstream of $tRNA_4^{Thr}$ as inferred from the results of Taylor and Burgess[14]. The Pribnow box (□) is preceded by a −35 sequence (TTGCAT) similar to the consensus sequence TTGACA of Rosenberg and Court[53] and also byan A+T-rich region (88% A−T from −63 to −38). A potential ribosome binding site[54], located 6 bases upstream of *tufB*, is underlined. The first 12 amino acids of *tubB* (ref. 19) are listed below their respective codons.

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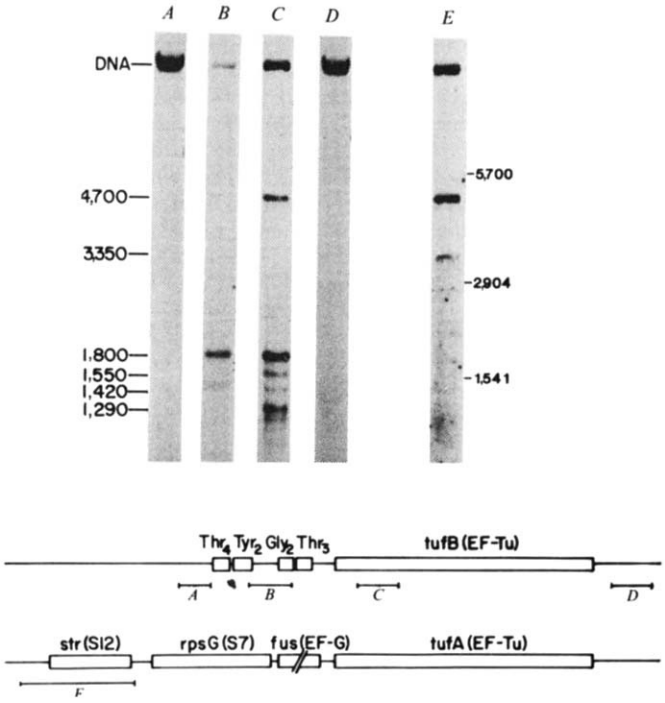

Fig. 2 Characterization of the transcript specifying the *tyrU* cluster and *tufB*. *E. coli* strain ABL-1 (RNase III⁻ RNase P^{ts}; ref. 55; gift of W. McClain) was grown for several generations at 30 °C, and then incubated at 42 °C for 30 min to inactivate RNase P. RNA was extracted according to Gegenheimer and Apirion[55] as detailed elsewhere[30], denatured with glyoxal[56], electrophoresed in 1.5% agarose gels with recirculation of the 10 mM sodium phosphate, *p*H 6.5, 1 mM EDTA running buffer, and transferred to DBM paper[11]. The RNA blots were probed with DNA fragments (*A-E*) which were nick translated[57] with [α-^{32}P]dCTP (NEN; 2–3,000 Ci mmol^{-1}) to a specific activity of $\sim 5 \times 10^7$ c.p.m. per μg. Hybridizations were carried out in the presence of 10% dextran sulphate as detailed by Alwine *et al.*[11] but omitting carrier DNA. Blots were washed first in 0.3 M NaCl, 0.03 M sodium citrate, 0.1% SDS at 23 °C for 20 min, and then in 15 mM NaCl, 1.5 mM sodium citrate, 0.1% SDS for 45 min at 50–55 °C. The ^{32}P-labelled probes isolated from the region of λ rif^{d}18 depicted in the diagram were: *A*, *Hha*I-145; *B*, *Hinf*I-190; *C*, *Hinc*II–*Sma*I-187; *D*, *Bst*NI–*Hae*III-185. Probe *E* is a *Hae*III-530 fragment encompassing the S12 ribosomal protein gene, which was isolated from λ fus3 (obtained from J. Yates and M. Nomura). Molecular weight markers were 16S (1,541 bases), 23S (2,904 bases) and 30S RNA (~5,700 bases). Lanes *B*, *C* and *E* have ~2 μg RNA per lane, and lanes *A* and *D* have 30 μg per lane. The scale is in bases. Structural sequences for tRNAs and *tufB* are boxed.

shown in Fig. 4 for selected probes and detailed in the legend, each probe hybridized only to its corresponding λ rif^{d}18 restriction fragment.

The hybridization results place the promoter for the tRNA-*tufB* co-transcript directly before the tRNA$_4^{Thr}$ gene and the terminator closely following the *tufB* structural sequence. Note that these assignments are not precise, because a probe could share as much as 30 bases of homology with the transcript and fail to hybridize. Genetic evidence corroborates the location of the *tufB* promoter directly upstream of the tRNA cluster[60]. In addition, Taylor and Burgess[14] found that a restriction fragment containing the presumptive tRNA-*tufB* promoter (labelled *Hae*III-890 by Taylor and Burgess[14] and *Hae*III-700 by Rossi *et al.*[15]) formed filter-retainable complexes with RNA polymerase. Finally, inspection of the DNA sequence reveals a promoter region situated directly before the tRNA$_4^{Thr}$ gene (see Fig. 1), a location consistent with the analyses of the functional promoter. The location of a possible termination sequence 34 bases following *tufB* (ref. 16) is also consistent with our hybridization results (Fig. 3). That termination occurs at this site *in vivo* is also suggested by the presence of gene *U*, which is transcribed from its own promoter ~200 bases downstream of *tufB* (refs 16, 17).

Other EF-Tu transcripts

EF-Tu is encoded by duplicate genes that contribute approximately equivalent amounts of EF-Tu to the cell[18,19]. The two proteins differ only in the carboxy-terminal amino acid[19], suggesting that they are functionally equivalent. The other gene for EF-Tu, *tufA*, is located at 72 min as the terminal gene of the *str* ribosomal protein operon[13]. The *str–rpsG–fus–tufA* genes are transcribed from a single promoter[13] located 80 bp upstream of *str* structural sequence[20], which could yield a primary transcript with an estimated size of 4,800 bases.

Probes prepared from *tufB* structural sequences hybridize to both *tufA* and *tufB* transcripts, as the DNA sequences of *tufA* and *tufB* differ at only 13 of 1,181 bp[16,21]. The largest *tuf* transcript was suspected to be *tufA* specific, as probes immediately upstream and downstream of the tRNA-*tufB* region failed to hybridize to it (Fig. 2). A probe isolated from the 5′ end of the *str* operon was used to prove that the 4,700-base transcript encoded the *str* operon. The *str* probe hybridized to the 4,700-base transcript that was also detected with the *tufB* structural probe (Fig. 2 *E*), indicating that the 4,700-base transcript corresponds to a full-sized *str* operon mRNA. This same probe also hybridized to a 3,350-base message that did not hybridize with *tufB* structural probes and could therefore encode the first three genes of the *str* operon (*str–rpsG–fus*). Our present data do not indicate whether the three smallest RNAs (1,290, 1,420, 1,550 bp (originate from *tubB* or *tufA* or both. As determined by densitometric tracings, these small RNAs are less abundant than the major 1,800-base transcript (by 2-, 22-, and 6-fold for 1,290-, 1,420- and 1,550-base transcripts respectively).

Modes of EF-Tu synthesis

The presence of one copy of EF-Tu in a ribosomal protein operon and the other in a tRNA operon may be important in coordinating the synthesis of these three components of the translational machinery. The intracellular concentration of EF-Tu does, in fact, parallel that of tRNAs and ribosomes during changing growth conditions in *E. coli*[22–24]. Moreover, EF-Tu synthesis from both loci is subject to stringent control in response to amino acid starvation[23,25–28].

In addition to transcripts corresponding to full-size *str* operon (4,700 bases) and tRNA-*tufB* operon (1,800 bases), there are three smaller *tuf* messages (1,290, 1,420 and 1,550 bases) (see Fig. 2). The 1,290-base message corresponds to the non-tRNA cleavage product of the 1,800-base primary transcript as shown in Fig. 5. In addition, an RNA of this size could result from transcription initiating at a promoter site (TGATGTC) located 32 bases upstream of *tufB*. Indeed, some promoter activity has been observed in this region *in vivo*[60]. The intergenic region also contains a possible termination sequence (CAACAA; refs 29, 30) that overlaps a sequence (TTCAACAAGTC) postulated to be involved in processing because of its homology with the characterized *tyrT* processing site (ref. 31; see also Fig. 6). Due to this overlap the alternative, non-mutually exclusive pathways that could uncouple EF-Tu synthesis from that of the tRNA cluster (processing versus termination and re-initiation of transcription) cannot be distinguished. However, the presence of large amounts of the 1,800-base transcript (Fig. 2) indicates that termination following the tRNA cluster (if it occurs at all) cannot be very effective in these growth conditions.

A similar potential for uncoupling exists at the *tufA* locus. The 3,350-base *str–rpsG–fus* message (Fig. 2) could result from either a termination or processing event or both. Analogous to the *tufB* region, promoter activity of DNA segments directly upstream of *tufA* has been observed *in vivo* (ref. 21 and J. D. Friesen and co-workers, personal communication). As noted below, the processing site conserved between the *tyrT* and *tyrU* transcripts is also present in the *fus–tufA* intergenic region, a finding that favours processing as a mode of uncoupling. Consistent with the suggested uncoupling of *tufA* synthesis, Nomura and co-workers find that translational repression of *str*

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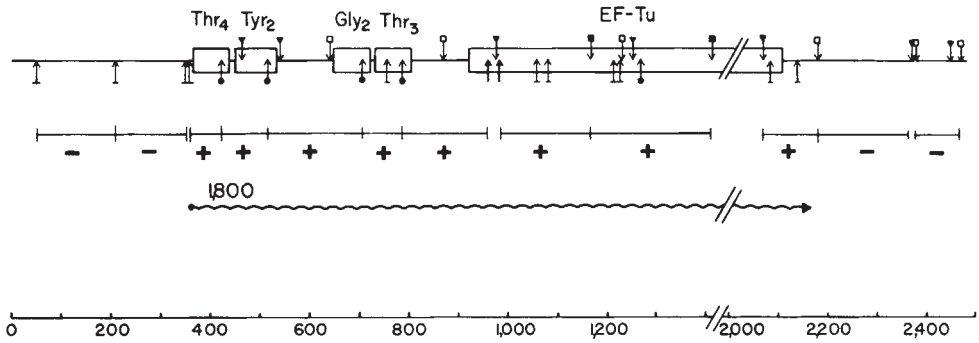

Fig. 3 Organization and transcription of the tRNA-*tufB* region. The locations of the restriction sites were obtained from the sequence shown in Fig. 1 or ref. 16. Hybridization to the 1,800-base transcript (⇝) is denoted by a + in the appropriate restriction fragment (from λrif^{d}18) and lack of hybridization by −. The *Hin*fI-82 probe from the tRNA$_3^{Thr}$ region hybridized strongly to 16S RNA in addition to the 1,800-base transcript. This result is consistent with the presence of a number of 12–16-bp homologies between 16S RNA[58] and tRNA$_3^{Thr}$ sequences. Due to the hybridization with 16S RNA, the *Hin*fI-82 probe could not be used to determine whether the smaller *tuf* transcripts (1,550 and 1,420 bases shown in Fig. 2) were *tufA* or *tufB* specific. The scale is in bases. Structural sequences for the tRNAs and *tufB* are boxed. ⚲, *Bst*I; ↓, *Hae*III; ↑, *Hha*I; ⚲, *Hinc*II; ↑, *Hin*fI; ⚲, *Sma*I.

operon by S7 protein does not inhibit EF-Tu synthesis *in vitro*[32,33] or *in vivo* (M. Nomura, personal communication).

Processing intermediates of the *tyrU* cluster

Figure 5 gives the tRNA processing intermediates of the 1,800-base primary transcript. Each probe hybridized to mature tRNA (75–85 bases), the 495- and the 1,800-base transcripts. Only tRNA$_4^{Thr}$ or tRNA$_2^{Tyr}$ probes hybridized to a 200-base transcript; similarly, only tRNA$_2^{Gly}$ or tRNA$_3^{Thr}$ probes hybridized to a 170-base transcript. The data suggest a processing scheme (depicted in the lower portion of Fig. 5) in which the primary transcript is initially processed to a 495-base transcript containing the tRNA cluster and a *tufB* mRNA of ~1,300 bases (Fig. 2). The tRNA precursor is subsequently processed to two dimeric precursors, a 200-base transcript containing tRNA$_4^{Thr}$–tRNA$_2^{Tyr}$ and a 170-base transcript containing tRNA$_2^{Gly}$–tRNA$_3^{Thr}$. Both these dimers are very close in size to those previously isolated from an *E. coli* strain with thermolabile RNase P activity[34–36].

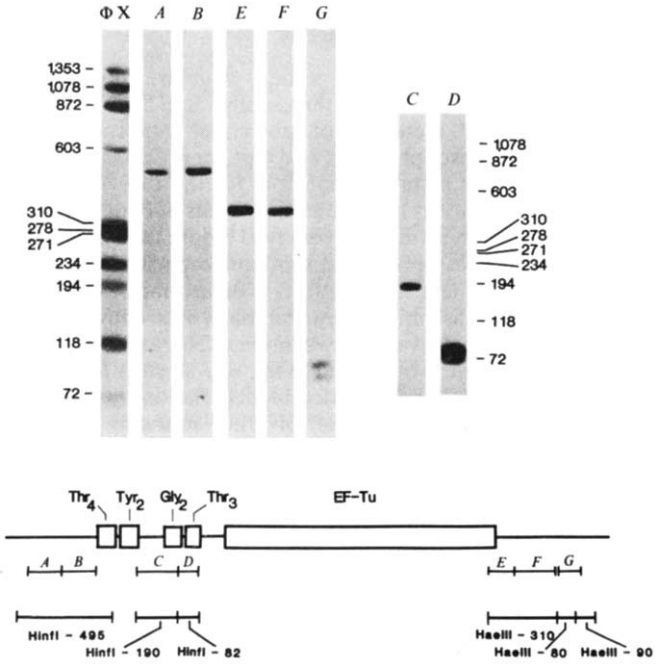

Fig. 4 Hybridization of probes to λ rif^{d}18 DNA blotted onto DBM paper. The *Bam*HI-*Eco*RI-5,500 fragment containing the entire tRNA-*tufB* region was digested with either *Hin*fI (lanes *A–D*) or *Hae*III (lanes *E–G*) and 0.1 μg of each digest per slot was electrophoresed on a composite gel (5% acrylamide, 0.7% agarose) cross-linked with *N,N'*-diallyltartardiamide as detailed by Alwine *et al.*[11]. The gel was soaked in 2% periodic acid to dissolve the cross-links before blotting. Blots were hybridized to the nick-translated probes illustrated in the diagram and washed as described in Fig. 2 legend: *A*, *Hha*I-150; *B*, *Hha*I-145; *C*, *Hin*fI-190; *D*, *Hin*fI-82; *E*, *Hae*III–*Bst*NI-125; *F*, *Bst*NI–*Hae*III-185; *G*, *Bst*NI-103. Probes *A* and *B* both hybridized only to the *Hin*fI-495 fragment (depicted below the probe) from which they can be isolated by *Hha*I digestion. Similarly, probes *E* and *F* each hybridized to the *Hae*III-310 fragment from which they can be isolated by *Bst*NI digestion. The two structural tRNA probes, *C* and *D*, hybridized only to their respective *Hin*fI-190 or *Hin*fI-82 bands. Importantly, the tRNA$_3^{Thr}$-specific probe (*C*) did not hybridize to the tRNA$_4^{Thr}$ fragment with which it has limited homology. Probe *G* hybridized to two *Hae*III bands ~80 and 90 bp, consistent with the existence of one *Hae*III site within the *Bst*NI-103 fragment and a *Hae*III site closely flanking each end (see Fig. 3). No hybridization was observed to additional bands when higher concentrations of DNA (0.5 μg per slot) or longer exposures were used. On the basis of the exposure times and DNA concentrations used in Fig. 3, the nick-translated probes can be expected to detect as little as 0.1 ng RNA. All the fragments (118–1,353 bp) of the ΦX174 *Hae*III digest (molecular weight marker) exhibited comparable band intensities when probed with ^{32}P-end-labelled ΦX174.

A secondary pathway for processing the tRNA-*tufB* transcripts may exist in RNase P-deficient strains. A 415-base transcript was apparent in lanes probed with *A*, *B* and *C* but not *D*, suggesting that this transcript consists of a tRNA$_4^{Thr}$–tRNA$_2^{Tyr}$–tRNA$_2^{Gly}$ trimer. This trimer may result from cleavage of either the 1,800- or 495-base transcript between the tRNA$_2^{Gly}$ and tRNA$_3^{Thr}$ structural genes.

The positions of the tRNA$_4^{Thr}$–tRNA$_2^{Tyr}$ and tRNA$_2^{Gly}$–tRNA$_3^{Thr}$ dimers, based on the size of the processing intermediates (Fig. 5) or obtained from the sequence of the tRNA$_2^{Gly}$–tRNA$_4^{Thr}$ dimer[34], are shown in Fig. 1. The long spacer separating the two dimers is similar to that found in *tyrT* (ref. 37) and *supB–supE* (ref. 38) and may be a common feature of tRNA clusters. The 114-base spacer separating the tRNA cluster and *tufB* is characterized by a 34-base C+T-rich region directly following the tRNAs.

The endonucleases responsible for cleaving the 1,800-base primary transcript have not been identified. One candidate was RNase III; however, there was no significant difference in the transcripts isolated from strains with or without functional RNase III (ABL-1 compared with A49). Other candidates include the tRNA processing endonuclease(s) partially purified by several laboratories[36,39–41]. It is of interest that the sequence conserved between the *tyrT* and *tyrU* primary transcripts, which is apparently the site for cleaving the tRNA cluster[31] from the distal message (see Fig. 5), is also present in the *fus–tufA* intergenic region[21] (TACCAAAGTC matches in 8 of 10 positions). The existence of a conserved processing site suggests that a single endonuclease may coordinately control EF-Tu processing from the *tufA* and *tufB* transcripts.

Finally, we note that in cells wild type for processing enzymes (CA274), the 1,800-base transcript is the only *tyrU* precursor detectable; none of the tRNA processing intermediates can be seen. Furthermore, as the amount of 1,800-base transcript in CA274 is comparable with that observed in cells deficient for processing (RNase P^{ts} or the double mutant RNase III$^-$ RNase P^{ts}), we suggest the tRNA sequences on the 1,800-base transcript are matured and function as tRNA in the cell.

Generality of dual function transcripts

The presence of tRNA on the same transcript as mRNA may not be rare in prokaryotes. An analysis of the *in vivo* transcripts of the tRNA$_1^{Tyr}$ gene in *E. coli* has revealed a 650-base transcript

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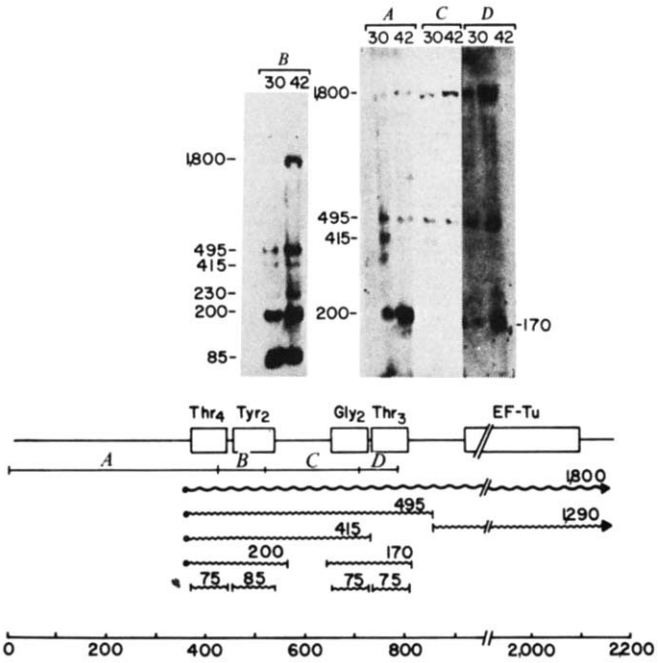

Fig. 5 Processing intermediates of the *tyrU* cluster. RNA was extracted from *E. coli* strain A49 (RNase P^{ts}; ref. 59; gift of W. McClain) grown at either 30 or 42 °C, electrophoresed on 2% agarose gels at 30 μg RNA per slot and blotted onto DBM paper. Transfer conditions were similar to those described for Fig. 2 except that glyoxal was removed with a lower concentration of sodium hydroxide (10 mM compared with 50 mM for Fig. 2). Molecular weight markers were 5′-end-labelled *Hae*III and *Hinc*II fragments of ΦX174. The gel shown in *B* was electrophoresed for a shorter time to retain mature tRNA. The ^{32}P-labelled probes isolated from the regions of λ rif^{d}18 illustrated in the diagram were: *A*, *Hin*fI-420; *B*, *Hin*fI-93; *C*, *Hin*fI-190; *D*, *Hin*fI-82. The putative primary transcript (⌇⌇), processing intermediates and mature tRNAs (⌇⌇) are labelled by their respective sizes (in bases) on denaturing gels. The probe containing the structural tyrosine tRNA sequence (*B*) hybridized to a 230-base transcript that also hybridized with a tRNA$_1^{Tyr}$ upstream probe and must therefore be a *tyrT* transcript. Probe *C* contained only 18 bases of structural tyrosine tRNA sequence, which severely limited its binding to the 200- or 230-base tyrosine transcripts in the stringent washing conditions used in these experiments. In the blot shown, the hybridization of probe *C* to the 170-base transcript did not photograph; in other blots this probe detectably bound the 170-base transcript. The processing scheme is discussed in the text.

which, in addition to containing two tRNA$_1^{Tyr}$ sequences, can code for a small arginine-rich protein[30]. The putative basic protein is encoded by the first of the three repeated units that follow the tRNA$_1^{Tyr}$ structural gene[37]. That the *tyrT* transcript actually encodes protein is suggested by the *in vitro* production of a low molecular weight, basic protein that maps to the first repeat[61].

Transcripts of the *tyrT* and *tyrU* gene clusters share several interesting features (Fig. 6): (1) the 5′ portion of both transcripts consists of two regions of cloverleaf secondary structure that are separated by a long spacer (115–208 bases), (2) the tyrosine structural sequences present on each transcript are almost identical, differing by only two bases in the variable loop region[42], and (3) following the tRNA region there is an intergenic spacer of 67–114 bases containing a conserved sequence (TTCAAAAGTC; located in a region of dyad symmetry) which seems to be the site for processing the tRNA cluster from the mRNA portion of the transcript (ref. 31 and Fig. 5). Finally, the proteins encoded by the two transcripts may share the property of being produced in large quantities by the cell. EF-Tu represents as much as 5% of the total cell protein in *E. coli*[22,24,43,44]. The basic protein encoded in the *tyrT* transcript, if actually a DNA-binding protein as suggested by its resemblance to the basic protamines[61], might also be expected to be relatively abundant.

Co-transcription of tRNA and mRNA has also been observed in other systems. In mitochondria, where transcription initiates at a unique site, almost every protein coding sequence is directly flanked by a tRNA gene[45–47]. Although the co-transcript seems to be very unstable, this arrangement may provide a requisite economy of space in the genome and/or conveniently placed processing signals in the nascent RNA[45,47]. Another system is bacteriophage T4, where a cluster of eight tRNAs is transcribed from the *ip*I promoter located 1,000 bases upstream[40,48].

Physiological implications

The tRNA secondary and/or tertiary structure could stabilize adjacent mRNA by retarding the usually rapid 5′ to 3′ degradation of message[49]. Detection of substantial quantities of intact dual function transcript in cells wild type for processing enzymes is consistent with a stabilization role for the tRNA cluster. If mRNA stabilization is important, the results of Pedersen *et al.*[50] imply that the tRNAs must be removed before translation. They observed that decay of protein synthetic capacity for *tufA* and *tufB* differed by less than 20 (ref. 50). In these experiments, RNA synthesis was inhibited by rifampicin or streptolygidin and it is possible that cleavage between the tRNA and *tufB* mRNA was also impaired. This would mask any storage form of the transcript that could be activated for translation by cleavage of the proximal tRNA. Alternatively (not mutually exclusive), co-transcription of tRNA and mRNA may be viewed as a mechanism for coordinately controlling the synthesis of key proteins with those of stable RNAs, thereby regulating the production of a particular protein with the growth requirements of the cell.

Conclusions

Analyses of the structure and transcription of the *tyrU* locus have revealed a new class of tRNA gene. Transfer RNA genes can be categorized by their proximity and co-transcription with

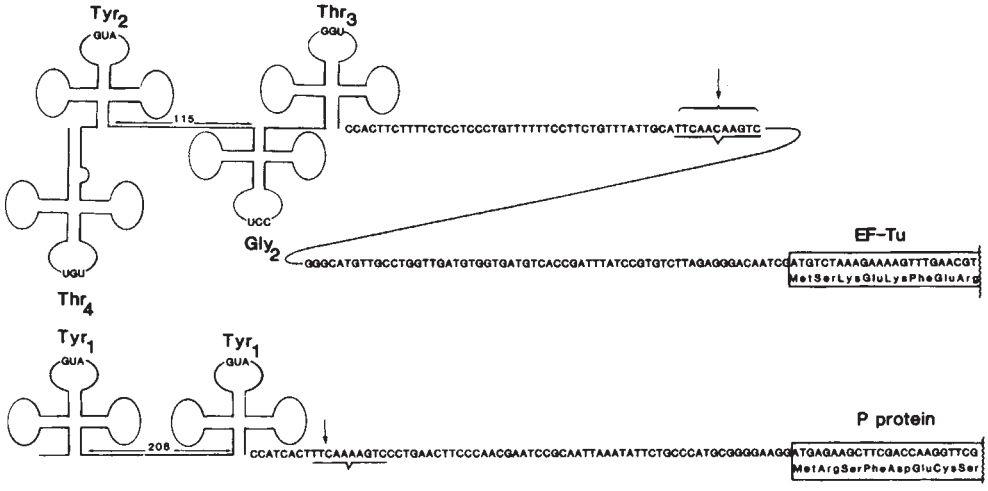

Fig. 6 Comparison of the *tyrT* and *tyrU* primary transcripts. The *tyrT* transcript was characterized by Rossi *et al.*[30] using RNA extracted from an RNase P^{ts} *E. coli* strain infected with Φ80 psu$_3^{+-}$ (doublet). The site conserved between the *tyrT* and *tyrU* gene clusters and known to be a 3′-processing site for *tyrT* transcripts[31] is underlined. For *tufB* (ref. 19) and for the protamine-like protein (P protein; see text), the first eight amino acids are indicated. Translation of P protein also initiates at the methionine located 4 codons upstream of the start site shown, as indicated by the *in vitro* production of both a 29- and 33-amino acid basic protein that map to the first repeat[61].

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either rRNA genes, other tRNA genes and/or genes encoding protein. It is tempting to speculate that the tRNA secondary and/or tertiary structure provides an additional level of post-transcriptional control for dual function transcripts. The precise role of the tRNA molecules on the co-transcript remains to be defined.

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LETTERS TO NATURE

Recurrent Seyfert activity in spiral galaxy nuclei

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Hills[1] has pointed out that in some conditions black holes in galactic nuclei would rapidly grow by disruption and accretion of stars thus producing Seyfert luminosities ($>10^{43}$ erg s^{-1}). In this hypothesis only those galactic nuclei with a high central stellar density are susceptible to Seyfert pathology, and they evolve through only one Seyfert phase. Here, I suggest an alternative model for recurring activity in normal galactic nuclei based on the assumed presence of a massive nuclear black hole ($\sim 10^7 M_\odot$) and the observation of a very clumpy distribution of interstellar gas in the inner 200 pc of our Galaxy. The system of massive molecular clouds with low net angular momentum could provide an 'accretion event' every 10^7 yr with a duration of $\sim 10^5$ yr. In such a picture, most spiral-galaxy nuclei evolve through recurring Seyfert episodes of rather short duration; the 1% accretion duty cycle is roughly consistent with the fact that a few per cent of all spiral galaxies are Seyferts[2].

Molecular line observations of the central region of our Galaxy have revealed some 10^7–10^8 $M_\odot$ of interstellar gas in the inner 200 pc concentrated in massive molecular clouds (ref. 3 and refs therein). The 2.6-mm emission line of carbon monoxide has been particularly useful in mapping the distribution and kinematics of this molecular gas[4,5]. These studies show that there are essentially two kinematic groups of molecular clouds in the central region (Fig. 1). First, there are about 10 very massive clouds ($\gtrsim 10^6$ $M_\odot$) including the Sgr A and Sgr B2 complexes which lie entirely at positive longitudes and within a projected distance of 150 pc from the centre. These clouds have line-of-sight velocities which are generally permitted in the sense of galactic rotation but which are much lower than the circular velocity at the sub-central point[6]. Either these clouds are presently stretched along the line-of-sight towards the centre (an unlikely and short-lived configuration), or they are close to the galactic centre but do not take part in systematic galactic rotation—they have a relatively low net angular momentum with respect to the centre.

The second group of clouds seems to lie in a single kinematically connected feature with high systematic non-circular motion—the 'expanding ring of molecular clouds'[5,7,8]. This feature has been modelled by an expanding ring with a radius of 190 pc, a rotational velocity of 65 km s^{-1}, and an expansion velocity of 150 km s^{-1} (ref. 5). The total mass of this feature is between 10^6 and 10^7 $M_\odot$; therefore, the energy in expansion motion may be as high as 10^{54} erg. It has been suggested that the non-circular motion associated with this feature may be due to elliptical streaming in the presence of a bar[9]. While expanding features at large distances from the centre (such as the '3-kpc arm') may result from such elliptical streaming, it is less likely that the high non-circular motions within 200 pc of the centre have such a gravitational origin. The gravitational field in the inner few hundred parsecs is dominated by the bulge component of the Galaxy, and bulges seem to be predominantly axisymmetric systems. Kormendy[10] has reported that bulges in some barred galaxies are slightly elongated perpendicular to the outer bar structure, but the apparent expansion observed both in the 3-kpc arm and in the molecular ring would require pronounced elongation of the bulge in the same sense as the outer bar structure[11]. Therefore it is plausible that the non-circular motion of the molecular ring results from a burst of high luminosity or mass ejection from a central object.

If the ring consisted of non-interacting test particles it would

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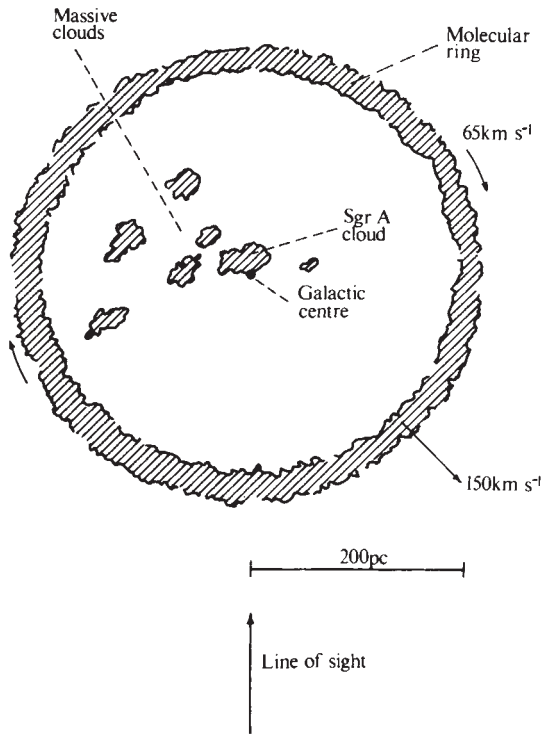

Fig. 1 The possible distribution of molecular clouds in the inner 200 pc of the Galaxy. The most massive clouds lie generally at positive longitudes and within the expanding 'molecular ring'. The Sgr A cloud may lie very close to the dynamical centre of the Galaxy.

oscillate between radii of 20 and 250 pc with a period of $\sim 10^7$ yr. But as the ring consists of gas clouds it would not survive one such period because it would encounter gas with very different kinematics during an oscillation (that is, the massive molecular clouds closer to the centre). Therefore to maintain such high non-circular motions in the molecular cloud region (and CO line observations of the nuclei of other normal spirals[12] do indicate the general presence of non-circular motions) a new outburst would be required every 10^7 yr or so, and this is the only evidence, albeit indirect, for recurring activity in our galactic nucleus. In terms of radiation from a central compact source, the centre of our Galaxy is certainly normal, but the high non-circular motions in the molecular cloud regions may be the hydrodynamic relics of short bursts of activity at the centre every 10^7 yr—relics which persist long after the other evidence of activity disappear.

We assume the presence of a black hole at the galactic centre with a mass $M_h \sim 10^7 M_\odot$. A compact object of this mass is entirely consistent with the velocity dispersion in the Ne II 12.8-μM line emitting clumps in the inner 1 pc of the Galaxy[13] and might be identified with the milli-arcsecond radio source in this region[14]. There are about 10 massive molecular clouds in the inner 150 pc of the Galaxy with individual masses of the order of $10^6 M_\odot$. If we consider these 10 clouds to be particles randomly moving in a cylindrical volume ($r = 150$ pc, $h = 70$ pc) then a cloud will encounter the black hole on a time scale of

$$\tau \sim \frac{1}{n_c v_c \sigma_c} \sim 10^7 \text{ yr}$$

where n_c is the density of clouds, v_c is the random cloud velocity (~ 200 km s^{-1} to support the system of clouds against the galactic gravitational field) and σ_c is the geometrical cross-section of a cloud determined from the observed cloud radius (~ 20 pc). The clouds would also encounter one another on about the same time scale and, to maintain the random motions, the system of massive clouds would require an energy input similar to that required by the molecular ring.

A collision between a massive molecular cloud and a black hole could have spectacular consequences. The hole would accrete matter in a cylindrical column through the cloud; the radius of the column is essentially the Bondi radius:

$$r_a = \frac{GM_h}{v_c^2} \sim 1 \text{ pc}$$

The fraction of the cloud mass accreted would be

$$\frac{M_{acc}}{M_c} \sim \left(\frac{r_a}{r_c}\right)^2 \sim 2.5 \times 10^{-3}$$

and if $M_c \sim 10^6 M_\odot$ then the accreted mass is $M_{acc} \sim 2.5 \times 10^3 M_\odot$.

Converting this mass to energy with an efficiency of 10% would produce a total energy of

$$E \sim 5 \times 10^{56} \text{ erg}$$

Only a few per cent of this energy need go into gas motion to account for the observed non-circular motions of the molecular clouds—both the systematic motion of the molecular ring and the random motion of the massive clouds. Assuming axisymmetric accretion and neglecting the effects of radiation pressure on the cloud, the time scale for accretion would be roughly the time during which the cloud is in contact with the hole:

$$t_a \sim \frac{r_c}{V_c} \sim 10^5 \text{ yr}$$

If much of the energy came out in the form of radiation then the mean luminosity would be

$$L \sim E/t_a \sim 10^{44} \text{ erg s}^{-1}$$

Therefore, every 10^7 yr, a cloud–black hole encounter would produce a burst of high luminosity lasting 10^5 yr; that is, a Seyfert nucleus would be turned on for $\sim 1\%$ of the time.

An extremely clumpy distribution of low angular momentum gas in nuclear regions of spiral galaxies implies a highly variable accretion rate onto a massive nuclear black hole. The fraction of time in which accretion is going on is equal to the volume filling factor of the gas, and in the inner 200 pc of the Galaxy, this is a few per cent.

Hills[1] and Ozernoy[15] have objected to the existence of a massive black hole in the galactic centre on the grounds that stars would be consumed too rapidly and thus produce a luminosity much higher than the observed upper limit to the luminosity of a non-stellar central point source. Assuming that the stellar density in the inner 1 pc core of the Galaxy is $\sim 10^7 M_\odot \text{ pc}^{-3}$, an upper limit of 10^{40} erg s^{-1} (ref. 16) on the luminosity of a compact source at the centre implies that the mass of a black hole must be less than $10^4 M_\odot$. It would be very fortuitous to see a black hole of this mass as the hole would grow to $10^7 M_\odot$ in only 10^9 yr; therefore a more probable upper limit to the black hole mass would be $100 M_\odot$ (refs 1, 15).

However, these arguments only apply if the mass of the hole is substantially less than the mass of the stellar system. If the black hole has grown to $10^7 M_\odot$ and dominates the gravitational field in the 1-pc core of the Galaxy, then the rate of accretion of stars is very much lower again due to the slow rate of orbit diffusion in a system dominated by a point mass[1]. In other words, the critical radius for 'loss cone diffusion'[17] exceeds the core radius of the nucleus. If f is the fraction of the core mass, M_c, in the black hole, then the rate at which the hole eats stars cannot exceed

$$\dot{N} = \frac{M_c}{m_* \tau_R}(1-f)$$

where m_* is the mean stellar mass and τ_R is the classical two-body relaxation time. With a 10% efficiency for converting consumed mass to energy, this implies that the luminosity due to consumption of stars is

$$L_N = 4 \times 10^{42} \left(\frac{M_c}{10^7 M_\odot}\right)^{1/2} \left(\frac{1\text{pc}}{R_c}\right)^{3/2} (1-f)^2 \text{ erg s}^{-1}$$

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which is less than the observed limit if $f \gtrsim 0.9$. A core mass of $10^7 M_\odot$ and a stellar system mass of $10^6 M_\odot$ (that is $f = 0.9$) are consistent with both the Ne II line observations of the core and the near-IR intensity of star light[13,18]; therefore consumption of stars by such a massive black hole in our galactic nucleus does not violate observational constraints. For galactic nuclei in general, accretion of stars may have contributed to the rapid growth of nuclear black holes at earlier epochs and the higher frequency of active objects at large redshifts.

The problem with the efficiency of accretion in a cloud–hole encounter is that, due to radiation pressure on grains, the cloud may be blown away on first contact or before all the matter in the accretion column has been captured. However, because of density gradients within the cloud the captured material would probably possess some net angular momentum with respect to the hole. Therefore passage of the cloud would result in the formation of an accretion disk. In this case significant accretion and production of energy could begin after the passage of the cloud and take place on a longer time scale than that estimated above.

The probable formation of an accretion disk suggests an observational consequence for the model. As cloud–hole collisions are a stochastic process, the accretion disk axis will have no preferred orientation with respect to larger-scale galactic structure. The jets observed in radio galaxies[19] and the low-luminosity radio jets recently observed in Seyfert galaxy nuclei[20] are probably produced and collimated on the size scale of the accretion disk ($\ll 1$ pc), possibly along the rotation axis of the accretion disk[21]. Therefore, in the context of the present model, jets produced in the nuclei of spiral galaxies should have no preferred position angle with respect to the galaxy, and, from the preliminary observations, this seems to be the case[20]. The radio jets observed in Seyfert galaxies have linear extents of a few hundred parsecs to a few kiloparsecs; therefore, the collimating 'device' must be present and stable for time scales of at least 1,000 yr which again is consistent with the time scale of the accretion event.

Most of the energy produced in active spiral nuclei appears as electromagnetic radiation whereas the power of active elliptical nuclei goes into highly directed bulk gas motion (jets). This striking difference is probably related to the mode of accretion. Perhaps the distribution of gas in ellipticals is much less clumpy and the resulting accretion flow is smoother[22]. This would suggest that the position angle of elongated radio structure in ellipticals should be more correlated with the overall galactic structure[23] or at least with the larger-scale gas distribution[24].

It would be of interest to observe the distribution and kinematics of dense gas in other spiral galaxy nuclei. In the context of this model, one would hope to observe the two species of nuclear molecular clouds in other galactic nuclei— clouds with systematic inflow or outflow and massive clouds with a low net angular momentum with respect to the galactic centre. The volume filling factor of such clouds should be of the order of a few per cent. High-resolution molecular line observations must await the development of millimetre-wave interferometers, although observations of the global kinematics of nuclear H II regions (the 'hot spot' nuclei) may also be relevant.

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Soft X-ray imaging with a normal incidence mirror

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By sputtering[1,2], it is possible to make multilayered structures (layered synthetic microstructures or LSMs) with individual layers as thin as a fraction of a nanometre. Using these techniques, we have now made a multilayered interference mirror to reflect carbon K X rays ($\lambda = 4.48$ nm) at normal incidence. The structure consists of 76 layers of tungsten of thickness 0.765 nm with layers of carbon (thickness 1.510 nm) interspersed, deposited on a ⟨111⟩ silicon wafer substrate. This LSM was formed into a concave mirror of radius ~1.1 m by bending the substrate, and used in an optical set-up to form images of grids illuminated by an X-ray source. The mirror was found to have a resolution of 5 lines mm^{-1} and an efficiency, integrated over the C K band, of between 4 and 8%.

Until now, designers of X-ray optical instruments such as telescopes and microscopes have been forced to use mirrors operating at small glancing angles θ (θ = angle of incidence measured from the surface tangent)[3,4]. This is because the refractive index $n = 1 - \delta$ of all materials is close to unity throughout the X-ray region, so that the fraction of the radiation reflected at the interface between a homogeneous material and air (or vacuum) is very low for values of θ much greater than the critical angle $\theta_c = \sqrt{2\delta}$. Nickel, for example, has $\delta = 2.4 \times 10^{-5}$ at a wavelength $\lambda = 0.154$ nm, $\delta \approx 1.4 \times 10^{-2}$ at $\lambda = 4.48$ nm, and it can be seen that the corresponding reflectance values, roughly equal to $\delta^2/4$ for normal incidence, are extremely small.

LSMs operate in a manner exactly analogous to multilayer dielectric coatings at visible wavelengths[5], and can be used to

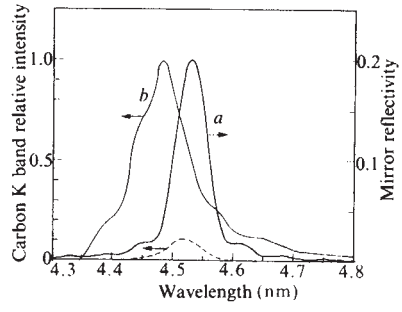

Fig. 1 Reflection of X rays by an LSM consisting of 76 layer pairs of tungsten ($d_W = 0.765$ nm) and carbon ($d_C = 15.10$ nm). Curve *a* (right-hand scale) is the reflectivity computed for the structure using the following optical constants: tungsten $\delta = 1.10 \times 10^{-2}$, $\beta = 1.29 \times 10^{-2}$; carbon $\delta = 1.30 \times 10^{-3}$, $\beta = 1.86 \times 10^{-4}$. Curve *b* (left-hand scale) is the relative intensity of the C K-emission band[8]. The dashed curve (left scale) is the product of curves *a* and *b*. The area under this curve is 6% of that under curve *b*.

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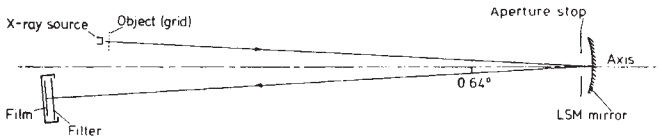

Fig. 2 Optical set up for normal incidence imaging with C K X rays.

increase the X-ray and UV reflectivity of surfaces. The resulting mirrors have numerous potential applications.

The simplest design is a periodic stack in which layers with a relatively high value of δ (material A, thickness d_A) are alternated with layers of low δ (material B, thickness d_B). Such a structure reflects X rays with intensity maxima at the Bragg angles θ_m given by the relation

$$m\lambda = 2(d_A + d_B)\sin\theta_m \quad (1)$$

where m is the order of reflection. (Note that this simple relation must be corrected for the effects of refraction and absorption[5,6].)

We have constructed an LSM to reflect the K band of carbon ($\lambda = 4.48$ nm) in first order at normal incidence ($\theta_1 = 90°$), and have used it to obtain images of a grid illuminated with a carbon target X-ray tube. The structure consisted of 76 layer pairs of tungsten ($d_W = 0.765$ nm) and carbon ($d_C = 1.510$ nm) deposited on a silicon wafer with ⟨111⟩ surface orientation, 76.2 mm diameter and 0.38 mm thick. Figure 1 shows the reflectivity of the structure as a function of wavelength, computed using a standard multilayer program[5,6] using values of δ and β (absorption index) given by Henke and Lee[7]. The relative intensity of the K-emission band of graphite carbon is also shown[8]. Although the peak reflectivity predicted for this multilayer structure is 20%, the layer period is not optimum for maximum reflectivity of C K radiation. The dashed curve in Fig. 1 is the product of the LSM reflectivity and the C K emission relative intensity, and represents an integrated reflectivity of ~6%. Note that any errors in the refractive indices will introduce uncertainties in the reflectivity calculations, affecting in particular the peak position.

The LSM-coated wafer was bent into a concave mirror of approximately spherical shape with a concentric-ring bending device patterned after that of Wassberg and Siegbahn[9]. This mirror was then set up on an optical bench in a vacuum chamber in the configuration shown in Fig. 2. The test object was illuminated from behind by a simple Coolidge-type X-ray tube with a colloidal graphite-coated target 1.25 mm in diameter. A special fine-grained film sensitive to soft X rays[10], Eastman Kodak SO-212, was used as the detector. The film holder had a filter of 2-μm thick polycarbonate plastic foil coated with 120 nm of aluminium to exclude visible light.

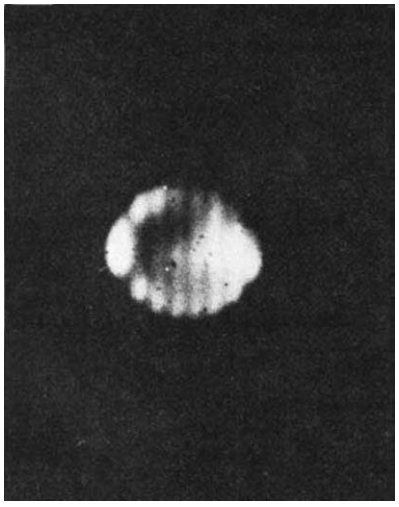

Fig. 3 Photograph of a 5 lines mm^{-1} grid illuminated by a 1.25 mm diameter X-ray source. The non-uniformity of the image reflects the non-uniform pattern of X-ray emission by the target.

The apparatus was first aligned and focused using visible light to illuminate the test object. It was found that the silicon wafer could be bent to a radius of ~1 m before severe aberrations set in. The object was set at a distance of 1,067 mm from the mirror and the film at a distance of 1,186 mm, with a resultant magnification of 1.11. As this off-axis configuration suffers from astigmatism, one-dimensional grids were used as targets and were set perpendicular to the plane of Fig. 2. The film was then set at position of the tangential focus.

Figure 3 is a photograph taken using C K X rays. The test object was an electroformed nickel grid with 0.1-mm bars separated by 0.1-mm spaces. The film was exposed for 1 h with an X-ray source current of 2.5 mA at 1.5 kV.

To reduce aberrations, the mirror aperture was stopped down to 7.6 mm. With this aperture, the bright strips are easily resolved: the target period of 5 lines mm^{-1} corresponds to about 40 arc s angular resolution. The resolution is limited by several factors, of which the most important is probably the imperfect optical figure of the mirror. A bending device of the kind used here bends even an isotropic lamina into only an approximation of a sphere, and in addition the wafer, being crystalline, tends to bend anisotropically and develop 'facets' that can be seen as a diffraction pattern in the visible light tests. Vibration of the optical bench, cantilevered in the vacuum chamber, is another possible source of degradation. Finally, the microscopic roughness of the layers themselves may cause scattering and degrade the resolution. This factor, which sets the ultimate limit of resolution that can be obtained with such a mirror, can be evaluated after the first two have been eliminated.

In this context, it is encouraging that tests with synchrotron radiation on similar LSMs yielded curves of reflectivity versus glancing angle in close agreement with those predicted from multilayer theory, indicating a high degree of layer perfection[2]. For the LSM described here, estimates of the reflectivity integrated over the C K band were made by densitometering an image and a piece of film exposed directly to the source behind a similar filter. The estimates lay between 4 and 8%, consistent with a 20% peak reflectivity and indicative of a close to perfect layer structure.

We conclude that normal incidence mirror elements for optical systems operating from a lower wavelength limit of 3–4 nm (soft X rays) out to 20 or 30 nm (EUV) are now feasible. Multilayer mirrors for these regions are being constructed both at Stanford University and at International Business Machines Corporation (see refs 11, 12). These optical systems have important potential applications in X-ray research and technology, and in some fields (for example, X-ray astronomy) may offer important advantages over glancing incidence optics, for example:

(1) The geometrical aberrations of normal incidence mirrors are much smaller, and the severe vignetting associated with glancing incidence mirrors is avoided thereby allowing a much larger field of view;

(2) Whereas a glancing incidence telescope reflects X rays of all wavelengths down to a certain cutoff value, the radiation reflected by an LSM is essentially monochromatic, so that auxiliary filters are not required;

(3) The collecting area of normal incidence optics is much greater for a given polished area of mirror (or substrate). Hence LSM optics, when fully developed, will probably be more cost effective.

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Satellite measurements of H_2O fluorescence in the mesosphere

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The concentration of water vapour in the mesosphere and above cannot be easily measured because the quantity of water is very small due to the low atmospheric density. Now, however, we report the observation of resonant fluorescent radiation from water vapour in the 50–95 km region by the Stratospheric and Mesospheric Sounder (SAMS) on Nimbus 7 satellite. Our preliminary data suggest that the mixing ratio of water vapour decreases with increasing height over this region with a value of $\sim 1\times10^{-6}$ v/v at 75–85 km.

There are believed to be two water vapour sources in the upper atmosphere, both of which are subject to tropospheric influences. The first is direct injection by mechanisms at present poorly understood but probably involving the Hadley cell circulation in low latitudes and the 'tropopause gap' in mid-latitudes; the second is indirect injection through methane, also originating in the troposphere, which is then oxidized to carbon dioxide and water in the middle stratosphere. Evidence for the latter process is given by the apparent decrease in methane concentration at these levels and a simultaneous increase in water vapour concentration[1].

The predominant sinks (see reviews in refs 2 and 3) are re-exchange to the troposphere and photolysis in the upper atmosphere followed by the escape of the hydrogen produced

$$H_2O + h\nu \rightarrow OH + H \qquad (1)$$

Because water vapour influences upper atmosphere chemistry and hence the radiation balance and dynamics of the region, a knowledge of its concentration profile is important to our understanding of the upper atmosphere.

A multi-channel radiometer for composition sounding of the middle atmosphere, the SAMS[4], was launched on the NIMBUS 7 satellite on 22 October 1978. Various channels on this instrument are designed to sense CO_2, CH_4, N_2O, NO, CO and H_2O. The instrument distinguishes radiation from a particular gas by 'pressure-modulation', an emission-sensing technique which has been described in detail elsewhere[4].

To increase the signal the atmosphere is viewed in the limb (Fig. 1) which has the advantage that emission observations can be made from as long a path through the atmosphere as possible against the cold radiation background of space. Such a limb path possesses an air mass about 70 times that in a vertical path above the lowest point of the path. The atmosphere's pressure profile and the geometry of the path both weight the material in the path strongly towards the tangent point giving good vertical resolution ($\approx$3 km for an infinitesimal field-of-view). In contrast, the horizontal resolution of a limb path is rather poor ($\approx$400 km).

The fundamental fluorescence mechanism for the 2.7-μm water vapour band is excitation by sunlight at 2.7 μm followed by radiative decay to the ground state by emission of a photon of approximately the same frequency as that absorbed. However, competing effects can significantly influence or even dominate this simple process and of these effects three: collisional relaxation, indirect excitation and indirect radiative relaxation, need to be considered in detail. The details of the calculations will be published elsewhere.

The collisional relaxation rate of water vapour at room temperature has been measured[5] as $1.1\pm0.4\times10^4\ s^{-1}\ torr^{-1}$ for collisions with O_2, and $1.5\pm0.4\times10^4\ s^{-1}\ torr^{-1}$ for collisions with N_2. The radiative relaxation rate is $79\ s^{-1}$.

The unusually small ratio of the radiative to the collisional relaxation rate at one atmosphere ($\approx 8\times10^{-6}$ atm compared with, for example, the ν_3 (4.3 μm) band of CO_2 for which the ratio[6] is 2.6×10^{-4} atm) may be the most significant fundamental problem with this method of concentration measurement. It implies that the level at which collisional relaxation becomes important occurs very high in the atmosphere at a level of $\sim$90 km and the region of interest, the mesosphere, is a region of competition between collisional and radiative relaxation processes.

As the emission from the atmosphere in these conditions is almost entirely non-thermal it is necessary to compute the source function, J_l, at all levels before calculating the observed signal. Although in a general analysis account must be taken of radiative exchange within the atmosphere, in this case secondary scattering can be neglected and the source function for a plane-parallel atmosphere written in the form:

$$J_l = n_l B(\tilde{\nu}_0, T_l) = \frac{\beta_l F_s}{1+\rho_l} + \frac{B(\tilde{\nu}_0, T_l)\rho_l}{1+\rho_l} \qquad (2)$$

Suffix l refers to the atmospheric layer, $\tilde{\nu}_0$ is the central wavenumber of the band, T_l the kinetic temperature, B the Planck function and F_s the solar flux. ρ_l is the ratio of the collisional to radiative relaxation times, which depends on temperature and pressure. β_l accounts for radiative transfer within layers, absorption in the atmosphere above the layer and the structure of the 2.7-μm band; n_l is the factor by which the source function, J, exceeds the Planck function, B. The source function shows the same spectral dependence as the Planck function at temperature T_l. β_l is computed from a knowledge of the spectral line distributions using the AFGL line data compilation[7].

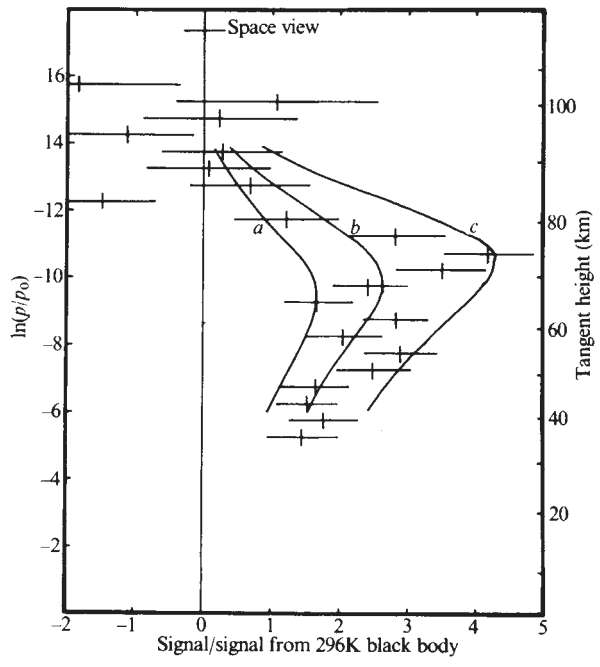

Fig. 1 The viewing geometry of the experiment. To keep the solar zenith angle to small values, data are only collected in the equatorial region. The satellite orbit height is $\sim$950 km.

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The most serious error in this equation probably arises from neglecting excitation in the 2.7-μm levels due to cascade decay from higher quantum levels, notably 101 and 011.

After computation of the source function the radiance measured by the instrument can be computed from a knowledge of the instrumental response function. For a pressure modulator radiometer this is a complicated function related to the lineshape and is non-zero only near the centres of spectral lines[4]. Because of this exact correlation between line positions and the instrument response function, the relative velocity of the atmosphere and the satellite must be included in the calculation. The instrument views the atmosphere in a direction perpendicular to the satellite velocity vector so that the only shift is due to the Earth's rotation. This induces a relative shift of about one doppler width in the signals and a 20% decrease in the observed signal.

Despite the enhanced emission due to fluorescence, the signals detected by the instrument are extremely noisy. This is due partly to the low density in this region and partly due to the apparently low water concentrations at these levels. Data for fluorescent measurements can only be taken when the satellite instrument is turned on, in a suitable operating mode and viewing the correct tangent height range[4]. The averages shown in Fig. 2 use all the available data from day 93 to day 103 1979, each point representing an average of 1,000–4,000 individual 1.8-s integrations. To eliminate effects caused by variations in the solar zenith angle, data are restricted to ±45° on either side of the equator. The vertical profile is quantized in units of 0.5 scale height (≈3 km). The nightside data were similarly analysed to demonstrate that this is a fluorescence phenomena and the signal in that region is zero as expected. The instrument is calibrated using the two-point method with a space view for zero signal and an internal calibration black body to provide a known radiance. The calibration procedure is more efficient than atmospheric viewing as the exact space angle is not critical and a reasonable instrument calibration can be achieved in ~36 h operating time.

The calculated signals shown in Fig. 2 have been computed from simple profiles of constant concentration with height using the data above for the collisional relaxation rate, assumed to be independent of temperature.

The most striking feature of the data is that they seem to predict a low concentration of water vapour in the upper mesosphere. The shape of the profile is fairly insensitive to the concentration of water vapour in the lower mesosphere and therefore the profile is reasonably consistent with either a constant, low concentration of water vapour or, more probably, a decreasing volume mixing ratio with height. Thus the shape of the profile is more consistent with that of Swider and Narcisi[8], who also show a decrease with height, than that of Arnold and Krankowsky[9], who show a constant mixing ratio with height.

Efforts are now being made to improve the quality of data reduction to obtain better information on the vertical distribution of water vapour as well as its seasonal variations.

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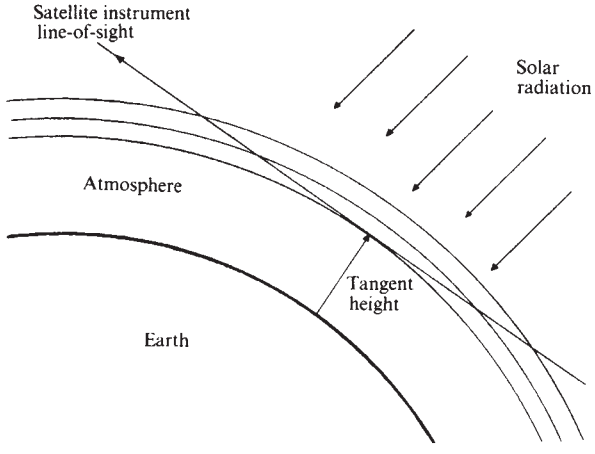

Fig. 2 Measurements and calculations for the spring of 1979. The three solid lines are calculated signals for constant volume mixing ratios of: *a*, 5×10^{-7}; *b* 1×10^{-6}; *c*, 2×10^{-6}. The data are shown by the crosses. The horizontal error bars are due to instrument noise and the vertical error bars to the vertical resolution of the averaging procedure. The zero radiance calibration point is established by periodically using a tangent height of >130 km, effectively eliminating all the atmosphere from the ray path. The standard deviation of the space view is shown as an isolated point at the top of the diagram. The vertical axis is $\ln(p/p_0)$ or the negative of 'scale height'. The tangent height scale on the right was constructed using an equatorial standard atmosphere for March.

Bond-lengths, bond angles and transition barrier in ice Ih by neutron scattering

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Although several studies[1,2] of ordinary ice (Ih) have established its nearly perfect oxygen arrangement and the statistical distribution of hydrogen among two symmetrically equivalent sites (the 'half-hydrogen' model[3]), several fundamental problems remain. The O—H bond length found in ice Ih is considerably larger than in other polymorphs of ice determined precisely[4,5], and many arguments have been put forward against the value observed[5,6]. Similarly, the tetrahedral H—O—H bond angle in ice Ih differs considerably from the value observed in the vapour phase, and this led Chidambaram[7] to propose a bent hydrogen bond model which further splits the atom positions, and which has not yet been verified. Finally, the mean shape of the double potential governing the atom distribution and the barrier governing the mobility still remain to be evaluated. We show here how high-precision, short-wavelength neutron diffraction data from ice Ih can be used to evaluate the molecular structure and atomic density distribution at 60 K. The unusually long O—H distance is confirmed, but there is no evidence for bent hydrogen bonds. Indeed the hydrogen atom density distribution is well described by a librational motion of the hydrogen atom around the oxygen. The mean barrier height between the 'half-hydrogen' atom positions was obtained from the scattering density as 0.012 eV, which implies that the zero point motion is of high importance for the proton exchange at low temperatures.

Measurements were done at the four-circle neutron diffractometer D9 of the Institut Laue-Langevin using a wavelength of 0.7107(5) Å on a pure specimen of 6.5 mm^3 cut from a larger sample (supplied by A. Chaillou, Laboratoire de Glaciologie, Grenoble). The temperature was maintained using a closed loop refrigerator and data were recorded with the ω–2θ step-scan technique. The intensities were reduced to structure factors using standard techniques[8], and corrections made during the analysis for absorption[9], anisotropic thermal diffuse scattering[10] and anisotropic extinction[11]. The cell dimensions used in

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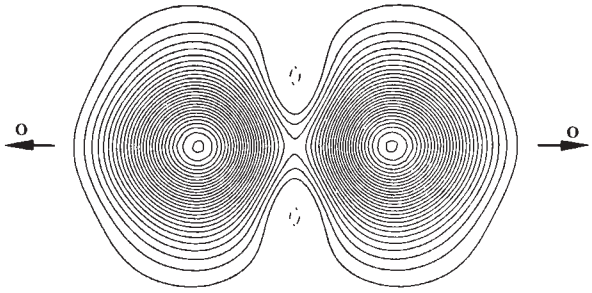

Fig. 1 Probability density map of H2 in the (0001) plane as described by harmonic and anharmonic terms up to sixth order.

the calculations are taken from the literature[12]: $a = 4.497(1)$, $c = 7.321(2)$ Å (1 Å $= 10^{-10}$ m). A total of 1,887 reflections were recorded up to $\sin\theta/\lambda = 1.07$ Å^{-1}, and they gave 288 reflections after averaging. The agreement factor among symmetry-related squared structure amplitudes was 0.041, and 222 of the final reflections had $I \geqslant 3\sigma(I)$.

From the data, different structural models were tested using crystallographic least-squares procedures. All the calculations were done using the Prometheus-system[13], which allows incorporation and refinement of higher-order thermal tensors. The 'half-hydrogen' model was refined successfully to a weighted reliability factor R_w of 0.013. Introduction of anharmonic thermal parameters (using a Gram–Charlier expansion of the harmonic trivariate gaussian probability function[14]) improved the agreement to $R_w = 0.012$. To estimate the double well potential correctly from the scattering density, a calculation was carried out with the hydrogen atom located midway between the oxygen atoms, that is fixed on the saddle-point of the double-well potential. If terms in the expansion up to the sixth order were included the refinements were successful. Only one hydrogen atom at a time was refined this way, and the two agreement factors obtained for H1 and H2 were 0.015 and 0.018, respectively. The results of all refinements show clearly the disorder of the hydrogen atoms. Figure 1 shows the atom probability density derived from the above refinements.

The results of a neutron diffraction experiment give scattering densities averaged over time and symmetry-related space in the crystal. If the molecular structure is represented conventionally by atoms undergoing harmonic motions, then observed interatomic distances must be corrected for the curvilinear relative motions of the atoms. This correction is not straightforward, as the curvilinear motion cannot be separated from the other atomic motions. However, using an anharmonic probability density function description of the hydrogen atoms allows a direct calculation of the true mode–mode distances as well as their, possibly different, equilibrium–equilibrium distances. The resulting bond lengths and bond angles given in Fig. 2 confirm the earlier observations, and indicate fundamental differences between the electronic hybridization state of ice Ih and that of

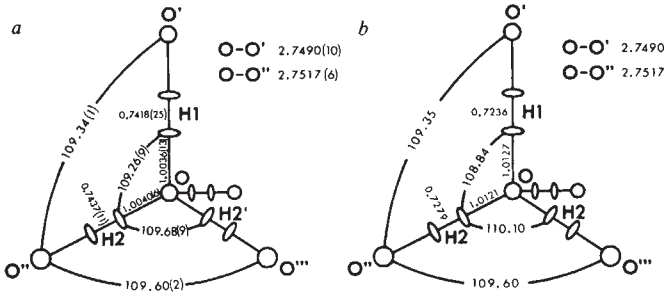

Fig. 2 *a*, The mean–mean bond distances and angles in the harmonic approximation. The errors (given in brackets) include the errors of the lattice constants. *b*, The mode–mode bond distances and angles of the anharmonic treatment. The corresponding equilibrium–equilibrium values are almost identical at a temperature of 60 K. The errors are hard to calculate and therefore not given.

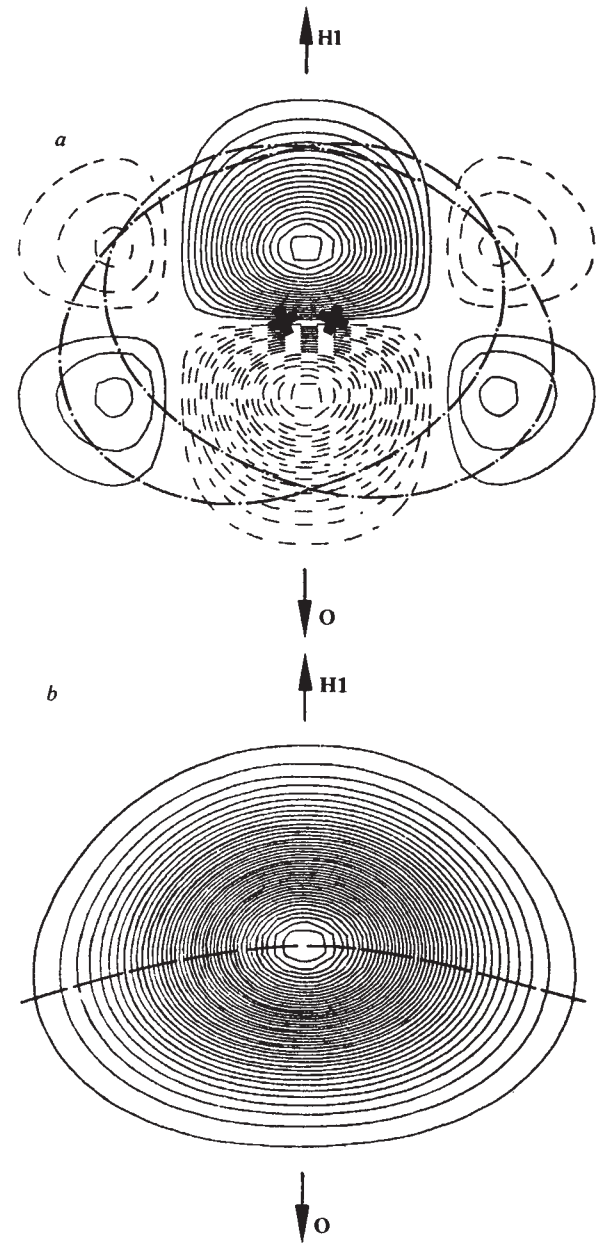

Fig. 3 *a*, Skewness-map of H1 in the $(10\bar{1}0)$ plane. Solid contours show positive, dashed contours negative modifications of the harmonic (gaussian) density distribution. The dashed-dotted lines give the equiprobability contours of two atoms of the bent-bond model; their equilibrium sites (exactly in-plane) are marked by crosses. *b*, Total probability density map of H1 in the same $(10\bar{1}0)$ plane as Fig. 3. The librational motion is clearly visible; the dashed line shows the trajectory of this motion. Note the similarity of this density distribution to a superposition of the bent-bond model densities given in Fig. 3*a*.

other ice phases studied. This could probably be caused by the complete hydrogen disorder coupled with the very low transition barrier as discussed below.

Further structural refinements were done testing the 'bent-hydrogen bond' model[7] by introducing the corresponding sixfold hydrogen splitting. The resultant agreement factors were comparable with those for the refinements of the anharmonic model. The outcome is, however, less favourable for two reasons. First, a comparison using Hamilton's R-factor test[15] and starting in both cases from the harmonic model showed the improvement to be highly significant on the 0.005 level for the anharmonic model (one parameter added), whereas the bent-hydrogen bond model improvements were only significant on the 0.05 level with three parameters added. Second, one would

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Table 1 Root-mean-square displacements of the harmonic model

Atom	r.m.s.d. (Å)	Orientation of principal axes: Angle with a_1	Angle with a_2	Angle with c
0	0.1166 (4)	90.0	90.0	0.0
	0.1176 (11)	—	—	90.0
H1	0.1335 (12)	90.0	90.0	0.0
	0.1787 (8)	—	—	90.0
H2	0.1360 (11)	90.0	36.3	111.5
	0.1762 (11)	90.0	108.5	158.5
	0.1801 (11)	0.0	120.0	90.0

expect the hydrogen probability distribution to show signs of modes, or at least a positive modification in the direction of the bending of the bond, and this is not the case. Figure 3*a* shows the deformations from harmonicity found for one of the hydrogen atoms with the equiprobability contours of the bent-bond model superimposed. The main feature in the deformation density is clearly derived from the librational motion of the atom, whereas in the direction of the expected bending the density is merely slightly modified and as expected in the opposite way. Thus the librational motion has a slightly preferential orientation only and can be described as a motion on the surface of a sphere. A section through the total density is given in Fig. 3*b*. The bent-bond model resembles this motion enough to mimic the density at the price of placing fractional atoms on weakly marked saddle-points in the density. The bond angle of 105° can therefore be ruled out noting, however, that the largest motion is in the 'soft' direction corresponding to the proposed bend.

The atomic root-mean square displacements (r.m.s.d.) are given in Table 1. The expected stretching frequencies calculated from the thermal motion in the harmonic approximation are 950(38) cm^{-1} for libration and 3,780(380) cm^{-1} for O—H stretch, which agree well with other experimental values[16].

There was some evidence for a measurable probability density between the 'half-hydrogen' positions in the Fourier maps given by Petersen and Levy[1]. This is confirmed by the present calculations, and clearly observable in Fig. 1. The density at the saddle-point is about four times the estimated error, and corresponds to a barrier height of ~0.012 eV for both hydrogen atoms. These low barrier heights imply both that even at low temperatures the transition frequency is high and that the zero-point motion must be of importance in the proton transfer process. From the full mean potential as derived from diffraction data one can estimate transition probabilities and lingering times, and this work is now in progress together with further studies at other temperatures and of the deuterated species.

This study confirms the earlier observations[1,2] of the geometry of the water molecule, namely that the H—O—H angle is 5° larger than in the vapour phase, and that the O—H bond is considerably larger than the vapour-value of 0.97 Å (ref. 6). Whereas it is quite common to observe increases of 2–5° in the H—O—H angle in hydrated compounds, an O—H bond length in water larger than 1.00 Å is highly unusual. As argued by Whalley[6] this would correspond to a storage of elastic energy which is one-fifth of the sublimation energy or several times larger than the energy difference between ice Ih and ice II (ref. 4) or ice IX (ref. 5), which have both been observed to have mean O—H bond lengths of 0.98 Å. Likewise[6] the observed change in frequency for the O—H stretch does not reflect the large change in the bondlength when compared with similar results in ice II and ice IX. The present measurements of the mean structure cannot by itself be used to give an explanation of these differences, but some indications can be sought from the detailed description of the atomic density distribution (see Fig. 1). Here there is clearly a finite probability of finding H in the middle of the bond. This would correspond to a transition frequency not much smaller than the stretching frequency. In the transition state, because of cooperativity (see, for example, the ice rules[3]), one hydrogen atom will be approaching oxygen while another is leaving and this will create an entity with an electronic state much different from normal H_2O, but undoubtedly with all O—H bonds longer than in water. The diffraction result is a sampling over all states with sampling times shorter than the transition time, and the result is an observed elongation of the O—H bond. Both ice II and ice IX are ordered structures, and one would thus, even without a detailed analysis of the scattering density, not expect a similar behaviour. Only very extensive quantum chemical calculations and lattice dynamical estimates would give the actual increase in bond length and indicate why the effective transition barrier is so low. Based on the present measurement we can only state that there seem to be coherence between the observation of the long O—H bond distances and the low barrier.

The list of structure factors is available from the authors.

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The role of liquid halogen in the reaction of crystalline KBr with gaseous Cl_2

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Problems in formulating a mechanism[1,2] for the reaction of crystalline KBr with gaseous Cl_2 ($KBr(s) + Cl_2(g) \rightarrow KCl + BrCl$) (a nucleation and growth process) and the role of dislocation proliferation[3] in promoting interface advance have been widely discussed. The evidence that local textural changes, in the vicinity of an active reaction interface, could be observed microscopically prompted us to examine the system using scanning electron microscopy. From these textural observations together with kinetic and other measurements, we conclude that liquid halogen (Cl_2, BrCl and Br_2) and dissolved intermediates, contained within the pore system permeating the solid product assemblage (the nucleus), are essential participants in the chemical change. We believe that reactant KBr dissolved in condensed halogen retained in pores at the reaction interface then interacts with chlorine to form polyhalide intermediates (probably including $[BrCl_2]^-$) subsequently depositing KCl product. Microscopic observations show that the nucleus is composed of KCl plates, penetrated by narrow (~0.2 μm) channels. This reaction model could be applicable to other systems, as previous discussions of solid state nucleation and growth processes have only rarely explicitly considered the possible participation of an intranuclear liquid and dissolved intermediates[4,5].

A feature of the reaction not emphasized in previous reports[1–3] was the invariable and strongly developed red-brown colouration of the product nuclei. This is important evidence of the retention of liquid bromine (and BrCl) within the product

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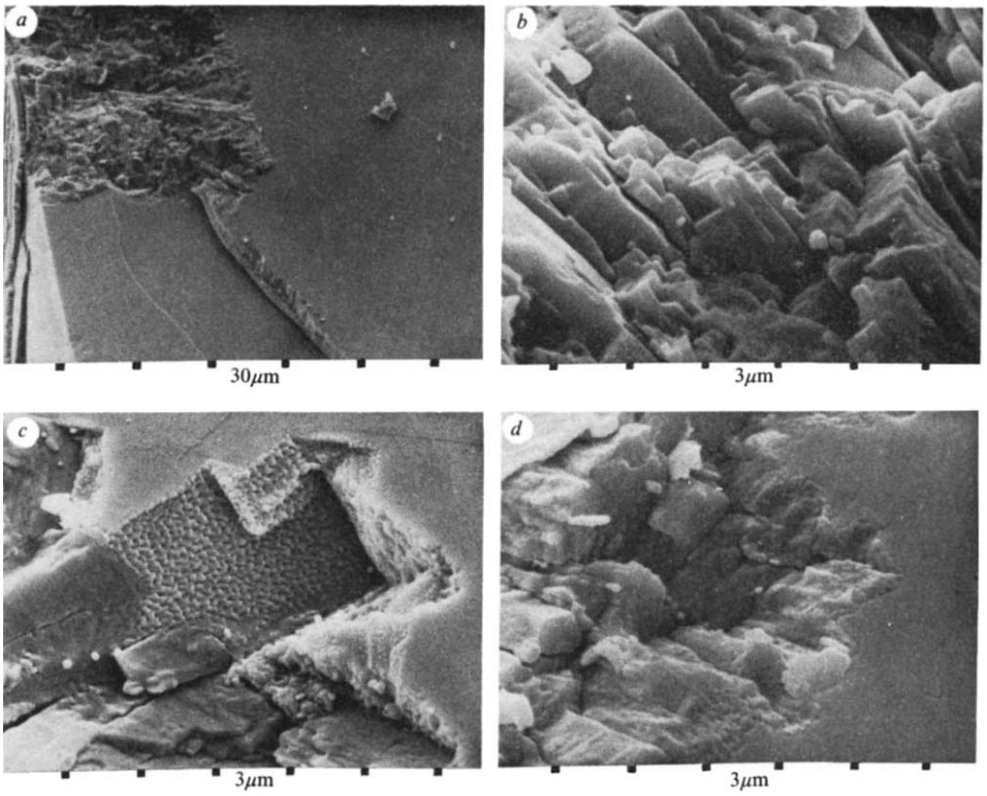

Fig. 1 Scanning electron micrographs of potassium bromide crystals after partial reaction in chlorine, cleaved in the (100) direction to reveal internal structures. Scales refer to spacing points at the lower edge. *a*, Cleavage across a nucleus; the unreacted crystal is smooth, the product is an irregular assemblage of small crystallites. Interface advance occurs in directions of principal axes. *b*, Texture of product within growth nuclei: KCl crystals and channels are oriented in the (111) direction (photograph edges are oriented in (100) and (010) directions). *c*, Details of interface after reaction at 273 K. *d*, Details of interface after reaction at 325 K.

assemblages which, on standing in air or vacuum at ambient temperatures, volatilized as a brown, pungent gas. The free halogen content of recently reacted salt, which we measured by titration was 3% Br_2 by weight, though values varied between 1 and 5% depending on reaction conditions. This quantity of liquid would occupy perhaps one-tenth of the 13% volume diminution resulting from replacement of reactant KBr with product KCl. More importantly, this volume of gaseous halogen would not be accommodated within the pore space of the residual product. Differential scanning calorimetric measurements for the freshly reacted salt, over the 310–600 K temperature range, gave only a single small broad endotherm at 350–390 K, quantitatively consistent with the evaporation of this small quantity of Br_2, if present as a liquid. This is strong evidence of halogen retention during reaction.

Rate measurements were made using crystal fragments (0.5 × 5 × 5 mm) freshly cleaved from a single KBr block (Specac Ltd). The vacuum apparatus used was similar to that described in ref. 5. The KBr reactant specimen was maintained at constant temperature (±0.5 K) and, after initial evacuation (10 min at 10^{-5} torr), a known pressure (50, 100, 200 or 400 torr) of chlorine reactant was admitted. The cleaved KBr surface was observed with an optical microscope. Small nuclei were often approximately circular but after growth became square with bounding edges oriented parallel with the principal crystal axes. Predominantly diagonal cracking developed during continued growth. The red-brown colouration of the nuclei was usually most intense in the vicinity of the active reaction interface. Edge lengths of these square nuclei were measured at suitable time intervals to determine rates of interface advance. These were invariably constant—typically 0.02 $\mu m\, s^{-1}$ at 283 K. Rates increased with temperature by a factor of ~10 between 273 and 350 K. Growth rates for different nuclei on the same crystal face and for concurrent reactions within the same environment showed close agreement. There were, however, appreciable differences in growth rates of nuclei between successive experiments proceeding in nominally identical conditions. Rates of reaction increased with increase in the prevailing chlorine pressure. After interruption of reaction by evacuation, or a sudden reduction of chlorine pressure, the advance of the interface halted before continuation of reaction. We attribute this to immediate volatilization followed by slow replacement of the liquid halogen within the nucleus.

Previous work[1] has shown that induction periods preceding the development of growth nuclei were considerable (500–5,000 min at ~290 K) as were the subsequent acceleratory nucleation processes (perhaps half as long). The equation ($N = At^3[1-\exp(Ct)]$) has been used[2] to express the variation in the number of nuclei (N) present with time (t) (A and C are constants). We confirm the earlier observation[2] that nuclei are preferentially developed at sites of surface damage. We account for these kinetic characteristics by identifying potential nuclei as the finest superficial cracks within which there is condensation of halogen, BrCl and Br_2, released by slow reaction of chlorine with reactant surfaces, until a liquid droplet is formed. The subsequent promotion of reaction as a growth nucleus increases the availability of bromine, so accelerating the activation of further nucleus-forming sites.

From the suggestion[2] that the nucleation process is catalysed by specific catalysts, we reasoned that such a substance would be a covalent liquid, capable of dissolving halogens at reaction

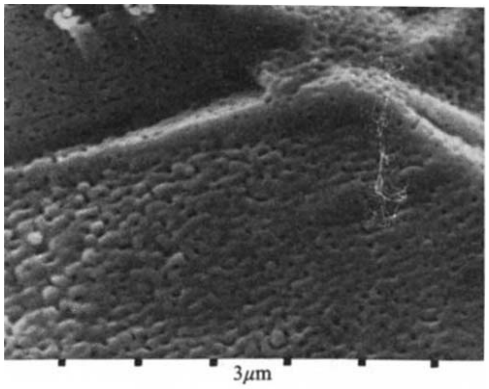

Fig. 2 Scanning electron micrograph of potassium bromide surface after cleavage followed by exposure to saturated bromine vapour for 5 min at 288 K. Cleaved surfaces were planar with step edges and exposure to bromine vapour (or liquid) caused modification including recrystallization which became more extensive after longer times of exposure.

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temperature (~270–370 K) and also form a weak complex or double salt with KBr and with KCl. Tin (IV) chloride possesses these properties[6] and a few small particles of metallic tin (20 μg) (rapidly chlorinated to $SnCl_4$ in reaction conditions) on the reactant KBr surface reduced the induction period to nucleation at 300 K from several days to <3 h. This catalysis by liquid $SnCl_4$ is further evidence for the participation of a liquid phase in this reaction. $GeCl_4$ and $PbCl_4$ similarly promoted the generation of growth nuclei.

Studies using the optical microscope were supplemented using the scanning electron microscope. Surfaces only could be investigated by this technique, though information concerning internal features of partly reacted crystals was obtained through the examination of surfaces exposed by cleavage after reaction. As reacted surfaces were particularly sensitive to water vapour, atmospheric exposure was minimized. Replication[7] of surfaces and pore structures of developed nuclei was not possible as textural reorganization of the reactant occurred in the solvents used.

Typical internal features of growth nuclei, as revealed by cleavage, are shown in Fig. 1*a–d*. In Fig. 1*a* the featureless area of unreacted KBr contrasts with the roughened texture of the section across the assemblage of product KCl crystallites. The rectangular development of nuclei is also evident, resulting from equal rates of interface advance in the (100), (010) and (001) directions. The higher magnification in Fig. 1*b* shows typical rectangular crystallites of product with intercrystalline channels oriented in the (111) direction (photograph edges are aligned with the principal axes). These channels contain liquid halogen during reaction, provide access for inward diffusion of Cl_2 to replace that which has reacted and permit removal of product Br_2 and BrCl. Characteristic textures of the irregular nucleus boundaries, the advancing reaction zone, are shown in Fig. 1*c*, *d*. There was no evidence that cracks penetrated the KBr beyond the nucleus. The etched appearance of the zone of active interfacial reaction (the centre of Fig. 1*c*) resembled textures developed on immersion of KBr in liquid Br_2 or on exposure to saturated vapour pressure of the gas; compare Fig. 1*c* with Fig. 2, which is a cleaved surface after exposure to saturated bromine vapour for 5 min at 288 K. Details of such surface retexturing varied with exposure times and temperatures. Retained bromine, released from nuclei on cleavage, could cause surface retexturing of adjoining zones of unreacted KBr. This again is evidence that liquid participates in the chemical change. Comparisons of Fig. 1*c*, *d*, for crystals reacted in 200 torr Cl_2 but at 273 and at 325 K, show that larger crystals of product were developed during the lower temperature reaction.

We conclude that the participation of a liquid phase in the interfacial processes provides a more satisfactory explanation than alternatives, such as strain, for several characteristic features of behaviour, such as the temporary cessation of interface advance after rapid gaseous reactant withdrawal, the activity of a liquid catalyst in accelerating the nucleation step and reactant textures at the interface. Furthermore, this involvement of homogeneous reactions within liquid generated in the vicinity of the interface is of wider interest because many studies have not characterized the chemical steps occurring within the reactant-product contact zone, which are often experimentally inaccessible. Some aspects of the forms and functions of nuclei have been discussed recently[5,8] but more often it has been assumed[4] that solid products do not impede the escape of volatile products. Much recent work has related rate data to the progressive changes of interface geometry as reaction develops. The identity of a rate limiting process is sometimes inferred from the magnitude of an apparent activation energy[4]. The participation of a liquid localized and temporarily retained at a specialist structure within the nucleus offers an attractive explanation of the reactant species mobility that may be required to permit a bond redistribution step. Furthermore, interface processes are often accompanied by recrystallization and retexturing which similarly may proceed more readily in the presence of a liquid. The elucidation of the chemistry of interface processes is a fundamental step in identifying the factors which control the reactivities of solids.

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Late Precambrian Keweenawan asymmetric reversals

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Palaeopoles from the Keweenawan (1,200–1,000 Myr) rocks of the Lake Superior region form the western and eastern arms of the 'Great Logan Palaeomagnetic Loop'[1–5] (Fig. 1*a*). This is defined by both normal (N) and reversed (R) poles which are the consequence of at least two geomagnetic reversals[4,6,7]. The younger one (R→N) has been detected throughout the Lake Superior region from various rock types.[1–6] The older reversal (N→R) is only recorded in the lowermost Keweenawan lavas in the southern part of the lake.[8] Wherever found, the reversals depart from the 180° symmetry (Fig. 1*a*)[2–6]. Due to these asymmetries, the Logan Loop has large segments that are devoid of data and thus the geophysical interpretations of the Loop (or 'hairpin') critically depend on the cause of the reversal asymmetry.[4] Four models[11] (secondary component[6], apparent polar wander[1–5], Wilson's dipole offset[5,7,9] and non-dipole field configuration[1,3,10]) have been put forward to explain the asymmetries. Here we demonstrate that the two-dipole field configuration model can explain the reversal asymmetries and discuss the global effects of this model in the light of worldwide late Precambrian palaeomagnetic data.

First we assume that the geomagnetic field during Keweenawan time consisted of an axial geocentric dipole (main dipole, $\vec{M}_{N,R}$) and an axial offset dipole ($\vec{m}$) (see also refs 12–14). The geocentric dipole has two polarity states, normal and reversed (Fig. 2). The magnetic dipole moments during these states are equal ($M_N = M_R$) but antiparallel ($\vec{M}_N$, $\vec{M}_R$). The field of the main dipole is disturbed by the persistent non-dipole (Nd) field component, the source of which is represented as the offset dipole ($\vec{m}$). The perturbing field represents a long-term zonal average of the Nd field[9,15]. An asymmetric reversal will take place, when the geocentric dipole reverses while the offset dipole retains its constant polarity.

In the first case the offset dipole with normal polarity ($\vec{m}$, $\vec{M}_N$) is located at the northern core–mantle boundary (Fig. 2*a*). If the ratio of the dipole moments (m/M) is 0.19, the inclinations at the Lake Superior area with a palaeocolatitude 53°, will be $I_R = -71°$ and $I_N = 42°$, corresponding to average values observed from Middle/Lower Keweenawan data (Table 1).

In the second case (Fig. 2b), the offset dipole of reversed polarity is located at the southern core–mantle boundary. If the ratio of the dipole moments is 1.20 (that is $m > M$), the inclinations at the Lake Superior area with palaeocolatitude 46° will be the same as in case I. However, in this case the ratios of the global average of the Nd field to the dipole field (d) are

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Table 1 Keweenawan palaeomagnetic data from the Lake Superior region (1,200–1,000 Myr)

No.	Rock types	Polarity	U/N*	D,I (deg)	Average intensity of NRM (10^{-3} A m^{-1})	Ratio of NRM intensities R_{Int}/N_{Int}	Palaeopole Lat., Long. (deg)	dp, dm (α95)† (deg)
1a	Duluth intrusives	N	7/97	289, 37	2240		33,192	(6.5)
1b	Duluth intrusives	R	1/13	91, −66	2440	1.09	42,200	(7, 10)
2a	Thunder Bay dykes	N	4/47	295, 43	5703		35,181	(9.1)
2b	Thunder Bay dykes	R	1/17	112, −68	4120	0.72	48,213	7, 8
3a	Coldwell Complex	N	1/7	294, 54	—		41,194	7, 9
3b	Coldwell Complex	R	1/13	119, −70	—	—	53,215	6, 7
4a	Upper Osler lavas	N	1/5	297, 40	2440		34,178	6, 10
4b	Lower Osler lavas	R	2/37	116, −61	8030	3.29	46,199	(18.4)
5a	Upper Mamainse lavas	N	2/66	296, 38	1240		33,182	(16.7)
5b	Lower Mamainse lavas	R	2/30	115, −72	1280	1.03	50,226	(41.6)
6a	Upper North Shore lavas	N	3/98	290, 45	2150		32,184	(4.3)
6b	Lower North Shore lavas	R	3/38	121, −63	1346	0.63	50,199	(11.0)
7a	Upper Gargantua lavas	N	2/14	295, 39	22		33,183	(12.2)
7b	Lower Gargantua lavas	R	2/11	132, −69	5728	—	59,213	(59.5)
8a	Lowermost South Range lavas	N	1/7	263, 37	3789		10,200	8, 14
8b	Lowermost South Range lavas	R	1/14	74, −72	2574	0.68	29,232	7, 8
Mean of normal data		N	8/350	290, 42	2927‡	1.24	32, 187	(7.7)
except 8a		N	7/343	294, 42	2754‡	(±1.02 s.d.)	35,185	(4.2)
Mean of reversed data		R	8/180	108, −71	3645		48,213	(8.6)
except 8b		R	7/166	115, −67	3824		50,209	(6.5)

* U/N = number of studies/sites.
† dp, dm = 95% confidence limits for the pole (single study); (α95) = 95% confidence circle for the pole (several studies combined). See ref. 7 for other refs.
‡ Excluding 7a.

unrealistically large ($Nd/d = 14$ (R polarity), $Nd/d = 1.5$ (N polarity)), whereas in case I the ratios ($Nd/d = 0.56$ (R polarity), $Nd/d = 0.38$ (N polarity)) are only about twice as high as those for the past 100 Myr (ref. 15). Note that due to axial symmetry of these models, the declinations have the observed 180° shift in both cases as observed[7] (Table 1).

If the main dipole now makes another reversal (R → N → R and so on), the inclinations will be successively asymmetric as observed in the Mamainse Point area[5], provided that the offset dipole retains its polarity. Because the palaeopoles are calculated by assuming an axial geocentric dipole field, the reversed poles (with steep negative inclinations) will be nearsided and the normal poles (with shallow positive inclinations) farsided with respect to the sampling site[9] (Fig. 1*a*). The true Keweenawan palaeopoles, assuming that no polar wander has taken place across the reversal, will be the same for normal and reversed epochs and lie roughly in the middle of observed poles, 53° (case I) or 46° (case II) from the sampling site. The true poles (TKM, TKL) according to our model I are shown in Fig. 1b.

If the offset dipole ($\vec{m}$) is of reversed polarity (case I) or of normal polarity (case II), the two models produce steep normal and shallow reversed inclinations. The distribution of

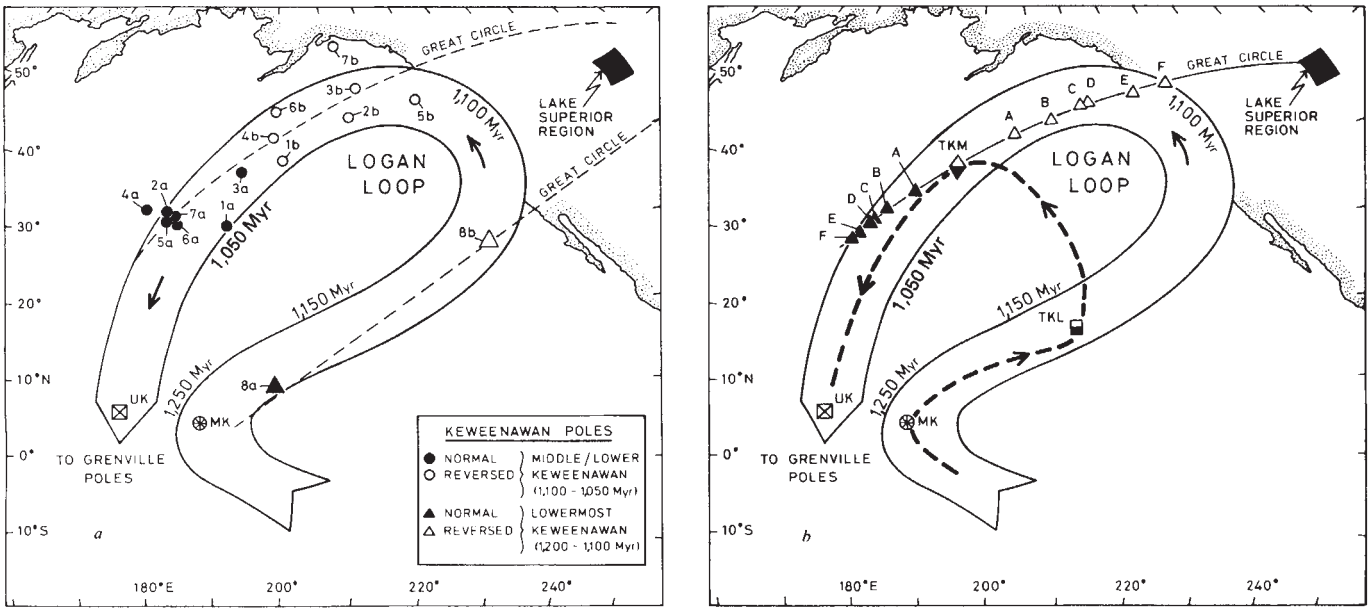

Fig. 1 *a*, The Great Logan Palaeomagnetic Loop as recorded in late Precambrian Keweenawan rocks from the Lake Superior region of North America. Only such Keweenawan data, where both normal (closed symbols) and reversed (open symbols) polarities are available, are indicated. The numbers and letters refer to Table 1. The great broken circles best fit the observed poles. The swath width (10°) corresponds to an average confidence value (α95) for an individual pole determination. MK, the average pole for the Mackenzie igneous interval (1,250 Myr); UK, the average Upper Keweenawan pole (1,000 Myr). For data and references, see Table 1 and ref. 7. *b*, The great circles and A–F represent the generated APW segments produced by the two-dipole model in Fig. 2*a* when the dipole moment ratio m/M varies from 0.10 to 0.30 (A, 0.10; B, 0.15; D, 0.20; E, 0.25 and F, 0.30). The point C ($m/M = 0.19$) corresponds to the average Middle/Lower Keweenawan asymmetry with $I_R = -71°$, $I_N = 42°$, respectively (Table 1). The poles TKM, (TKL) represent the true average Middle/Lower, (Lowermost) Keweenawan poles corrected for the two-dipole model. Note that there is no particular chronological order of poles from A to F in both polarities and the model allows APW 'jumps' back and forth along the generated polar wander segment. Such oscillatory APW movement has been observed in both Keweenawan[5,7] and coeval Grand Canyon[7,26] data.

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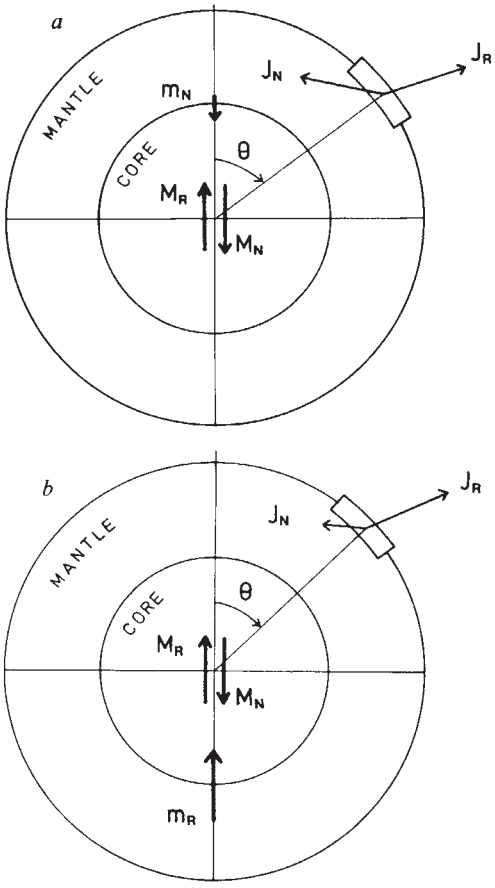

Fig. 2 Coaxial two-dipole geomagnetic field configurations which are capable to explain the late Precambrian Keweenawan asymmetries at the Lake Superior area. *a*, Case I model; *b*, case II model (see text).

palaeomagnetic directions of NE–SW trending late Gardar dykes of Greenland[16] may be interpreted in this way.

The distributions of Keweenawan poles in Fig. 1*a*, fit quite well onto two great circles (western and eastern), which intersect near the sampling site. This type of pole distribution is in accordance with the axial offset dipole models[7,12]. Therefore, the great depth extent of the Logan Loop may be a signature of the oscillation of the m/M ratio rather than plate motion as previously thought[1–4]. If we consider case I and allow the ratio m/M to vary 0.10 to 0.30 in steps of 0.05 while all the other variables remain fixed, the theoretical and purely apparent great circle polar wander segments can be generated as shown in Fig. 1*b*. The observed Middle/Lower Keweenawan poles (western arm) in Fig. 1*a* agree well with the theoretical poles. Note that the generated path segments of reversed and normal poles differ (Fig. 1*b*) because the function which maps the palaeomagnetic directions to the palaeopoles depends on the inclination, so that a longer segment of the palaeopoles is obtained for steeper inclinations[17].

In our new interpretation, the size and depth of the Logan Loop are drastically reduced. Plate motion during the Keweenawan is, however, still recognized in the new loop (Fig. 1*b*). The APW segments joining the Mackenzie pole (MK; 1,250 Myr) and the true Keweenawan poles (TKL, TKM) to the upper Keweenawan (UK, 1,000 Myr; ref. 7) and Grenville poles (950 Myr; ref. 18) probably represent the real paleomagnetic signature of the plate motion during the Keweenawan.

However, along the segment from the true normal pole (TKM) to the 'oscillatory' pole *F* (normal polarity), both plate motion and dipole oscillation are likely to cooperate in producing the APW swath. Note also that the reduced size of the Logan Loop decreases the previously proposed high APW rate.

Without the possibility of testing new models, they would be only *ad hoc Nd* explanations[19]. Three independent observations are available to test the credibility of our models.

The first test involves consideration of the distribution of the poles. The requirement that the Keweenawan poles be the consequence of the oscillation of the m/M ratio involves the assumption that normal and reversed poles lie on a great circle passing the sampling site[7,14]. As can be seen in Fig. 1*a*, this requirement is fulfilled for both arms within the limitations of accuracy of the available poles.

The second test involves palaeointensity data. The models shown in Fig. 2 predict the palaeointensity ratios of reversed and normal rocks at the Lake Superior area to be about 0.95 (case I) and 2.3 (case II), respectively. The observed averaged ratio of NRM intensities (Table 1) for six Keweenawan formations is 1.24 ± 1.02 (s.d.), with a range 0.63–3.29 (ref. 7). On the other hand, the average absolute palaeointensity ratio of reversed and normal units (only three formations studied) is 1.94 ± 1.64 (s.d.) with a range from 0.62 to 3.77 (refs 7, 20, 21). Thus the palaeointensity ratio varies so widely that we cannot define its average value well and we cannot, therefore, distinguish between case I, case II and the geocentric axial dipole case. More intensity studies with Thellier method, such as performed in the Thunder Bay district[7], are needed in the Lake Superior area to develop the two-dipole models.

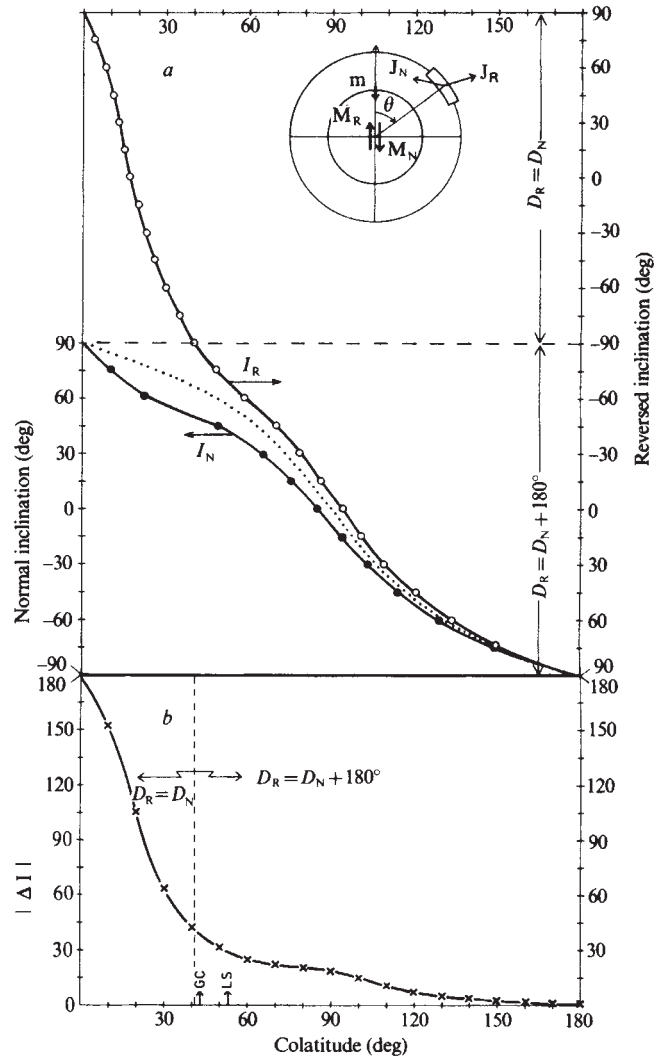

Fig. 3 *a*, The global values of inclinations due to the two-dipole model shown in the index figure (case I), as a function of colatitude θ. Open (closed) symbol for reversed (normal) data. The arrows (I_N, I_R) denote the average Keweenawan inclinations. Dotted curve is the axial geocentric dipole field inclination. *b*, The deviation $|\Delta I|$ from 180° of reversals of inclinations calculated from the data shown in *a*. The vertical broken line indicates the colatitude limit for which the declinations of normal and reversed remanences depart 180°. LS, colatitude of the Lake Superior area as measured from the true Middle/Lower Keweenawan pole (TKM in Fig. 1*b*); GC, corresponding colatitude of the Grand Canyon area.

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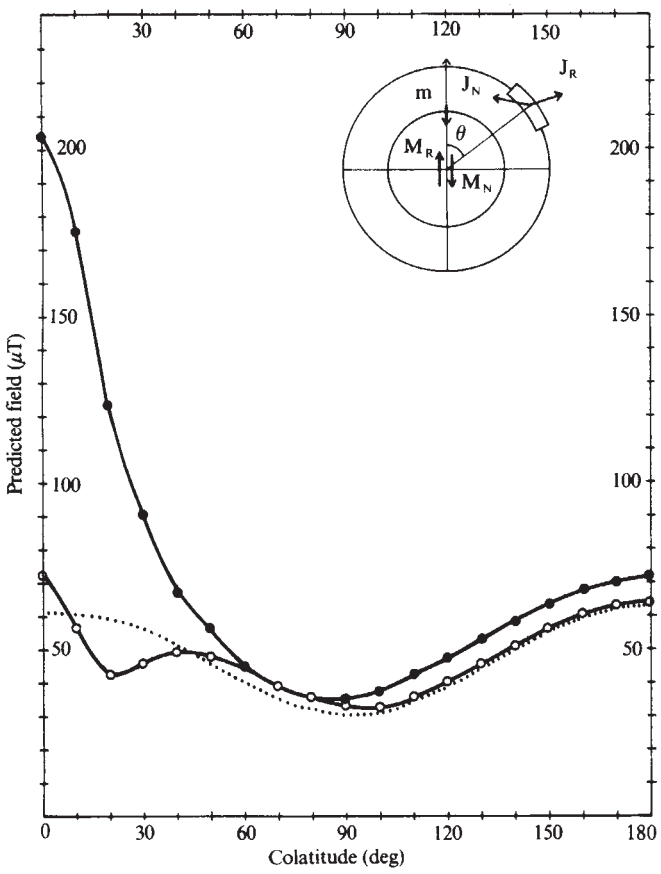

Fig. 4 The global values of field intensities due to the two-dipole model shown in index figure (case I), as a function of colatitude θ. Open (closed) symbol for reversed (normal) data. The curves are normalized so that at the palaeocolatitude 53° the intensity ratio of reversed and normal data is ~0.95 as produced by the case I model (see text). Dotted curve is the present Earth's magnetic dipole field intensity curve.

The third test involves a global study of field reversals of late Precambrian age (1,200–1,000 Myr). Figures 3 and 4 show the inclination and intensity values as a function of colatitude for normal and reversed epochs predicted by the two-dipole model (case I). It can be seen (for example, Fig. 3*b*) that asymmetric reversals should be observed at all colatitudes, and thus the feature is not restricted to the Lake Superior region. Particularly, strong asymmetry in inclination should be observed at colatitudes $\theta \leqslant 60°$. Note, however, that when the colatitude is smaller than ~40°, normal declination (D_N) becomes equal to the reversed one (D_R) (Fig. 3), and the term 'reversal' becomes confusing (see ref. 14). In case II the global asymmetries are much larger because the ratio of dipole moments is very large.

Unfortunately, the worldwide data[7] of Keweenawan age interval reveal only few studies where both normal and reversed data are documented. These data are of poorer quality than from the Keweenawan. However, in all of these cases (for example, Barby lavas in Africa[22], the Late Gardar dykes in Greenland[16], the Jacobsville sandstone in Canada[23] and the Grenville/Sveconorwegian rocks in Canada[18] and Scandinavia[7]) the reversals are always asymmetric. The inclinational asymmetry in these studies is 30° ± 18° (s.d.) while the corresponding declinational asymmetry is 19° ± 7° (s.d.) on average. Of particular importance and in support for our two-dipole model, are the palaeomagnetic data from Yenisei Ridge (1,200–900 Myr)[24], where several successive reversals are found, all of which are asymmetric. Another test will become available should the reversals[25,26] in the Grand Canyon area prove to be asymmetric also, because the Grand Canyon sequence is contemporaneous with the Keweenawan one[7,26]. The colatitudinal difference between the Lake Superior and Grand Canyon areas (relative to true Keweenawan poles) is ~10° and therefore the asymmetry, if it exists in the Grand Canyon, should agree with the curves in Figs 3 and 4.

We conclude that the oscillating co-axial two-dipole model can describe the available palaeomagnetic data of the Lake Superior area and suggests in particular that the puzzling asymmetric reversals of Keweenawan age may reflect persistent zonal *Nd* geomagnetic field components between 1,200 and 1,000 Myr.

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High-resolution seismic reflection profiles reveal fracture zones within a 'homogeneous' granite batholith

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Several countries are investigating the use of plutonic rock bodies for the ultimate disposal of radioactive waste material[1]. Among criteria[2,3] that must be met before a rock body is mined for a repository is that it must have a low *in situ* permeability. As the permeability of most unfractured plutonic rocks is very low[3], the problem reduces to finding those bodies which do not contain major connected fractures that would allow radioactive material to be transported by water to the biosphere. We report here that a high-resolution seismic reflection survey across the central region of an Archaean granitic pluton, which on the basis of other data might be defined as relatively homogeneous, identifies major sub-horizontal fracture zones at depth. Such fracture zones, which could be a major hazard for the containment of radioactive wastes, may therefore be detected before selection of a repository site by a combination of high-resolution seismic reflection surveying and suitably located deep drilling.

The Lac du Bonnet batholith is a 96 × 25 km pluton trending ENE–WSW in southeastern Manitoba, close to the border with Ontario[4]. This pluton is one of the crystalline rock bodies being studied at four research areas in the southern Canadian Shield by Atomic Energy of Canada Limited. It contains the proposed site of Canada's Underground Research Laboratory for conducting heating and other *in situ* scientific experiments in the subsurface.

Geologically, the Lac du Bonnet batholith is situated within the southern portion of the English River gneissic belt, a major Archaean belt within the western Superior tectonic province of the Canadian Shield (ref. 5; fig. 1). The central and major

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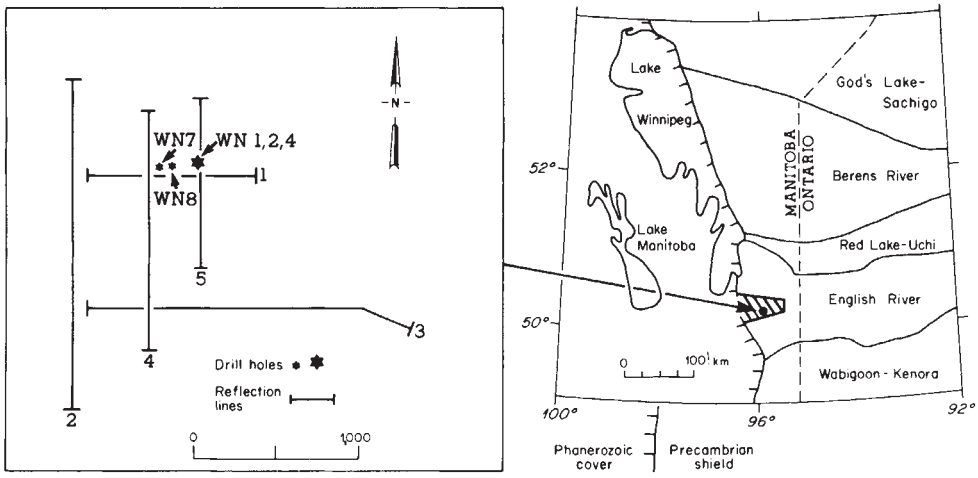

Fig. 1 Seismic survey lines across the Lac du Bonnet batholith. Inset shows the location of the surveys within the tectonic framework of the Canadian Shield[5]. Cross-hatched region outlines the approximate location of the Lac du Bonnet batholith.

portion of the batholith has been described as a homogeneous single phase of medium- to coarse-grained granite[4]. Minor heterogeneity is observed in the form of xenoliths of the surrounding country rock and pegmatite and aplite dykes. Economically important mineralization (such as the Tanco deposit[4]) is restricted to pegmatites that intrude the country rock near the northeastern end of the batholith[6].

Field evidence shows that the Lac du Bonnet batholith is intrusive into the adjacent formations and that the intrusion may have postdated the last phase of the Kenoran orogeny (~2,600 Myr) in this region[6]. This conclusion is supported by the fact that the batholith has undergone the least deformation of the rock units in the area[4]. In particular, there is only a small amount of deformation in the central region of the batholith, and this may be related to minor diapiric movement of the batholith after partial or complete crystallization.

The fractures and lineaments in the area of the batholith have been studied using satellite imagery, aerial photographs of two selected sites of 56 km^2 and ground observations at about 140 outcrops[4]. Although these studies showed three trends of fractures and lineaments (N–S, NE–SW and NW–SE), no major surface fractures were observed within the batholith. In a comparison with the lineament study by Brown and Thivierge[7] of 317 plutons larger than 3 km in Ontario, Tammemagi *et al.*[4] show a graph which we interpret as placing the Lac du Bonnet batholith in the top 3% of rock bodies in terms of structural integrity and percentage outcrop.

Several surface geophysical surveys have been conducted across the Lac du Bonnet batholith to delineate fractures and changes in lithology[8]. A N–S gravity traverse across the batholith and neighbouring formations reveals the low-density batholith at the centre of a negative regional gravity anomaly[9]. Towards the north end of the batholith, superimposed on the negative regional anomaly, is a shorter-wavelength positive anomaly that has been interpreted as due to a high-density body situated at a depth >4 km. Various density models[9] that fit these data indicate a relatively constant density distribution within the batholith above a depth of ~4 km.

Electromagnetic and resistivity surveys[10–12] across the central portion of the batholith have failed to produce any structural information, due to a widespread overburden layer of highly conductive clay, 0–25 m thick. Some evidence for lateral heterogeneities was obtained from a combined low-level (150-m flight altitude) magnetic total field and gradiometer survey[13]. The total field data show the magnetic field increasing smoothly from the boundaries of the batholith towards its centre. Near the centre, in the vicinity of the gravity high, are two particularly high-amplitude anomalies that have yet to be explained. The magnetic gradiometer survey revealed numerous anomalies across most of the batholith[13], but due to the lack of outcrop it has not been possible to test either the significance of these anomalies or relate them to specific lithological or structural changes within the batholith.

In 1979 and 1980, five high-resolution MINI-SOSIE [trademark of Société Nationale Elf-Aquitaine (Production)] seismic reflection profiles were recorded within a 2.2×2.2 km area of the south-central region of the Lac du Bonnet batholith (Fig. 1). The principal advantage of MINI-SOSIE over conventional seismic reflection systems is its inexpensive non-destructive energy source, consisting of one or more small Earth tampers[14,15]. In the field, 24 receiver arrays of nine 40-Hz geophones spread over 10 or 20 m were deployed at 10 or 20 m intervals. The seismic source provided a random series of pulses to the Earth and about 3,000 pulses, suitably time shifted and added, were recorded every 10 or 20 m to provide 12-fold common reflection point coverage. A split spread configuration was adopted and a digital rate of 500 samples per s was used for each 1 s seismic record.

Conventional seismic processing techniques (static and dynamic corrections, bandpass filtering, prestack sliding gain and common reflection point stacking) have been applied to give the two E–W seismic sections shown in Figs 2 (line 1) and 3 (line 3). The line 1 section has also been passed through a wide-band velocity filter (four input traces per output trace; passband −1.7 to +1.7 km s^{-1}) to remove prominent low apparent velocity arrivals associated with the overburden layer.

Figures 2 and 3 show that the Lac du Bonnet batholith is not as homogeneous in its central region as suggested by most other studies, but contains fractures and possible intrusions. There are at least two major sub-horizontal (dips < 25°) reflection

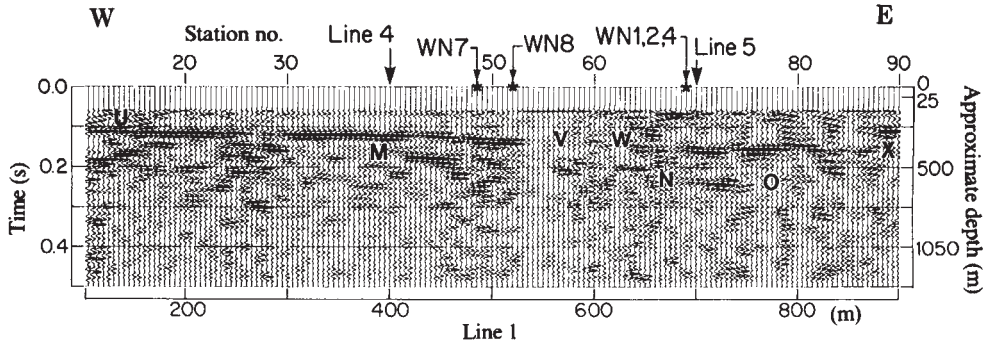

Fig. 2 Twelve-fold common reflection point seismic section collected along line 1 (Fig. 1). Lettered reflection zones are discussed in the text. The positions of the cross lines and exploratory drill holes are shown at the top of the section. Receiver spacing 10 m. Depths shown are only approximate due to the lack of detailed seismic velocity estimates throughout the region.

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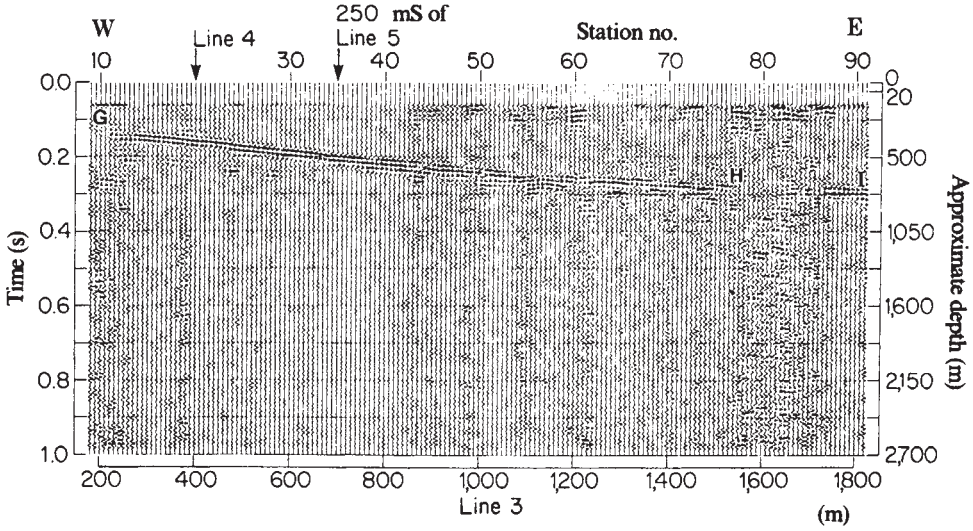

Fig. 3 Twelve-fold common reflection point seismic section collected along line 3 (Fig. 1). The position of the cross lines are shown at the top of the section. Receiver spacing was 20 m. Depths shown are only approximate due to the lack of detailed seismic velocity estimates throughout this region.

systems; UVWX in Fig. 2 and GH in Fig. 3. The true attitudes of these and other reflecting horizons are controlled by good-quality reflections on the cross-lines 1, 4 and 5 (Fig. 1).

The spectacular reflection zone GH (and possibly to I) on line 3 (Fig. 3) dips smoothly at an angle of 17° to the east and can be traced for a distance of 1.2 and possibly 1.6 km. On the N–S cross-lines this feature, which is represented by a series of strong reflections, has a more rugged character with a northward component of dip varying from 3 to 15°. It can be traced continuously from line 3 to line 1, where it is represented by the reflections MNO (Fig. 2). In the vicinity of line 1 this feature disappears and is replaced by the reflection system UVWX, which has apparent easterly and northerly dips of 8° and 9° respectively and can be mapped over the entire length of line 1 (Fig. 2).

Inclined drill holes near the cross-over of lines 1 and 5 (Fig. 1) have intersected major fracture zones starting at depths projected updip of ~360 and 600 m (ref. 16) which account for the reflections UVWX and MNO respectively in Fig. 2. The fractures near 360 m depth have been studied extensively by (1) standard geophysical logging techniques, (2) core logging (geological, geochemical and geophysical), (3) television logging[16], (4) hydraulic conductivity methods[17] and (5) tube wave analyses[18,19]. The television log confirms that they dip at shallow angles in a northeasterly direction, and the hydraulic conductivity and tube wave studies show that some fractures have high permeabilities. Preliminary analysis of data from hole WN7 shows that it has intersected the fracture zone UVWX at a depth of ~300 m, in excellent agreement with the depth predicted from the seismic data. Note that the drilling of WN7 near this depth disturbed the water level in holes WN1 and WN4 (C. C. Davison, personal communication).

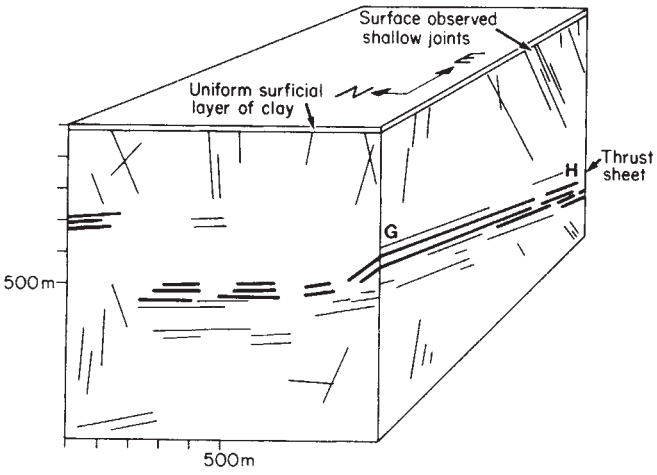

Fig. 4 Hypothetical block section of the Lac du Bonnet batholith viewed from the intersection of lines 3 and 4 (Fig. 1). A uniform (20 m thick) surficial layer of clay covers the batholith in this region. The sketch emphasizes the main features of the postulated thrust sheet G–H of Fig. 3.

We interpret the two reflection systems as originating from a pair of *en echelon* fault zones with a regional dip of 12° (UVWX)–18° (GH) along an ENE–WSW to NE–SW azimuth. Locally, the attitudes of the faults vary due to relief along the strike directions (Fig. 4). The projected surface outcrops of the fault zones lie beneath the Winnipeg River, and may have influenced the drainage pattern in this region. We suggest the original fractures developed with some relief along strike and movement was along the smooth shear planes (UVWX and GH in Figs 2 and 3 respectively) parallel to the dip direction.

The low dip angles of the fault zones are probably indicative of thrust faulting, caused by horizontal compressional stress in an ENE–WSW to NE–SW direction, approximately parallel to the long axis of the batholith. This stress field was not prevalent in Archaean times, during the batholith's intrusion[5], when the largely N–S compression of the regional stress field resulted in the E–W trending tectonic belts of the western Superior province (Fig. 1). However, the postulated ENE–WSW to NE–SW compressional stress field is parallel or sub-parallel to the current regional stress field inferred for much of northern North America. Focal mechanism studies of earthquakes in eastern Canada and the eastern United States show E–W to NE–SW deviatoric compressional and near-vertical deviatoric extensional axes[20,21], and this same stress field has been determined by hydro-fracture measurements[22] and with greater variability by *in situ* stress measurements[23,24]. *In situ* stress measurements at Red Lake, 120 km north-east of the Lac du Bonnet batholith, show predominant NE–SW deviatoric compression[24,25]. A similar NE–SW stress field has been suggested for sediments beneath the western Prairies of Canada, based on the preferred orientation of spalling in oil wells and the results of hydraulic and steam fracturing[26]. Although the E–W to NE–SW stress field may be a feature of much of the northern North American continent, the source of the stress may not be the same for the different regions.

This study of the Lac du Bonnet batholith, a rock body which on the basis of other data may be classified as relatively homogeneous, shows it to contain numerous fractures, some of which are interconnected and may reach to the surface. A hypothetical block section that might be viewed from the junction of lines 3 and 4 in Fig. 1 is sketched in Fig. 4. The high permeability and size of some of these fracture zones (UVWX is >50m thick and affects an area of at least 400×800 m) demonstrate that they may be a major problem in the search for a suitable repository site.

Our results indicate the feasibility of subjecting any potential repository site to a comprehensive high-resolution seismic reflection survey. A three-dimensional seismic reflection survey, combined with an appropriate exploratory drilling programme,

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could delineate blocks of a rock body that may be relatively unfractured and thus suitable for a nuclear waste repository.

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Bacterial origin of East Australian continental margin phosphorites

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Many models of the origin of marine phosphorites require a sediment rich in organic matter, which by decomposition releases phosphorus for the precipitation of carbonate fluorapatite[1–7]. This concept is useful in explaining contemporary phosphorite in areas of very high productivity off the coasts of Peru–Chile and South West Africa[8–10] where there are high organic matter fluxes to the sea floor[4–7,11]. It does not explain the origin of marine phosphorite deposits which formed in regions of limited oceanic upwelling and productivity[12–14]. The East Australian continental margin, an area of phosphogenesis throughout the late Pleistocene and Holocene[13,14], is a modern analogue of an 'East Coast' phosphogenic province[15–17] with low productivity over the upper slope region where the most recent phosphorites have been found[18]. Evidence is reported here for the carbonate fluorapatite in the East Australian phosphorites being located within bacterial cellular structures, and a model proposed for the origin of these deposits through the slow bacterial assimilation of phosphorus from seawater in an area of restricted sedimentation.

Phosphatic nodules and sediments were recovered by dredging and piston and box coring on the East Australian continental shelf and upper slope between 29° and 31° S at depths ranging from 210 to 450 m. In box cores, late Pleistocene to Holocene phosphatic nodules occur within unconsolidated sediments of silt to sand-sized quartz, glauconite pellets and planktonic foraminiferal tests. The sediments have a grain-supported fabric: the low concentration of fines is believed to have resulted from winnowing processes. Nodules of Holocene age (from uranium-series data) always occur within a few centimetres of the sediment surface. Nodules having uranium-series ages in excess of 240,000 yr often occur within the top 10 cm of the cores. This is consistent with a low sedimentation rate, or with significant mechanical reworking and erosion. Based on the uranium-series dating of three phosphatic nodules occurring at different depths in piston core P904 (Table 1), we interpret the distribution of nodules in the uppermost parts of the cores to be the result of a low sedimentation rate in the region. Assuming that the nodules formed at or near the sediment–water interface, the uranium-series ages indicate an average sedimentation rate of <1 cm kyr^{-1}. Table 2 summarizes geochemical data from six samples of nodules and sediments. The organic carbon concentrations are low compared with values reported for high productivity phosphogenic provinces. The phosphorus values for the sediments are also low. This suggests that carbon and phosphorus fluxes to the East Australian sediments are several orders of magnitude lower than those obtained from Peru–Chile and South West Africa[4,5,11].

Examination of freshly fractured surfaces of late Pleistocene to Holocene phosphatic nodules by scanning electron microscopy (SEM) with energy dispersive analysis revealed that the detectable phosphorus is located in structures morphologically resembling bacteria. The cellular structures are composed of apatite and resemble non-filamentous, botuliform bacilli 1.5–2.0 μm in length (occasionally as short as 1.0 μm) and 0.75–1.0 μm in width, generally cylindrical, but commonly with a tendency to be ovoid to subturbinate, with one end tapering to become more pointed than the other (Fig. 1*a*).

Together with fine clay minerals, the bacilli are concentrated in the matrix of the nodules, and occur attached to all solid surfaces including quartz grains, glauconite pellets and foraminiferal tests. Attachment of the cells to a surface is generally by the narrow end of the cell, but there is no development of a stalk. The morphology of the bacteria is seen most clearly on shell fragments where their numbers are small (Fig. 1*b*, *c*). There the bacilli did not form chains or mass into colonies, but appear to be randomly distributed, as if a motile cell had migrated to an anchorage. Where aggregations became greater, contiguous colonial growth occurs (Fig. 2*a*), and in some instances, dense bacterial colonies are layered with the long axes of the cells parallel to one another but normal to the supporting surface (Fig. 2*b*).

In contrast to the phosphatic nodules, the associated sediments are almost free of bacterial structures. Pyrite framboids are present in both the late Pleistocene to Holocene nodules and in the sediments in association with carbonaceous material; but they are not abundant, and where present usually occur within foraminiferal tests. Middle Miocene ferruginous phosphatic nodules from the same region contain few bacilli: the apatite is mostly present as well-developed crystals (Fig. 2*c*). In both the late Pleistocene to Holocene and middle Miocene nodules, sharp, well-defined X-ray diffraction peaks indicate good apatite crystallinity.

Given that all of the carbonate fluorapatite in the younger East Australian phosphatic nodules is present within bacterial structures, what evidence is available to help define the environmental conditions suitable for their proliferation, and identify their type? A consistent morphological feature shown in our micrographs is the relatively uniform size and shape of the bacilli, both within and between sampled sites. This suggests that we may be dealing with one specific organism, rather than with the range of forms that would signify a mixed population.

The absence of a mixed bacterial population indicates that the ecological niche occupied by this organism was highly restrictive, and possibly unique to a single species or group of closely related and morphologically indistinguishable species. The high degree of preservation of whole bacterial cells in the nodules suggests that they have been free from competition, predation and grazing. The most likely synopsis of these ecological condi-

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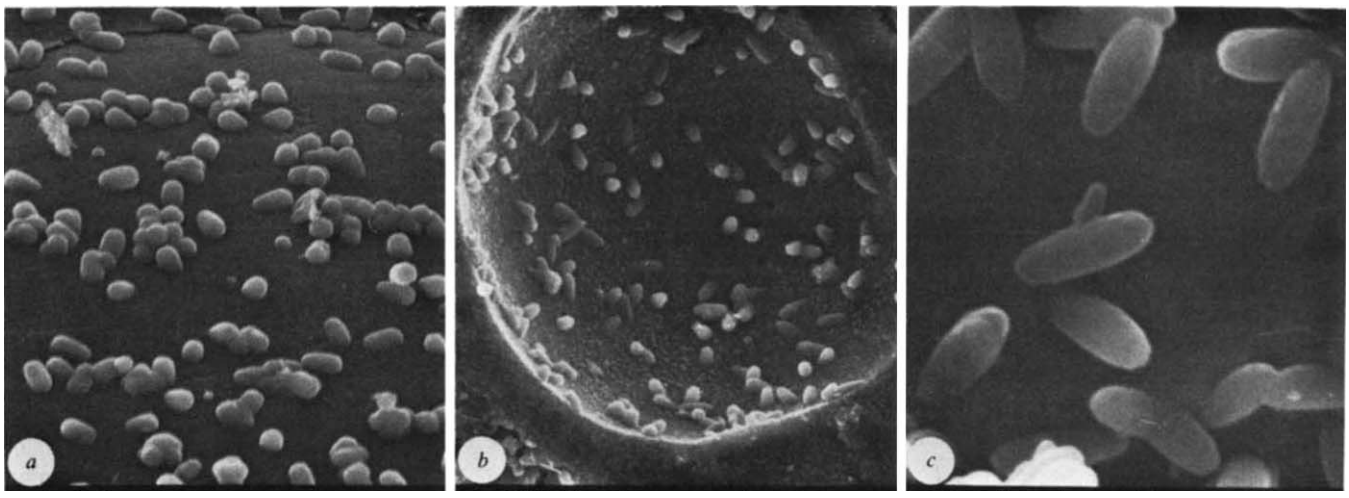

Fig. 1 *a*, Bacterial cells replaced by apatite encrusting a foraminiferal shell surface. Amongst the many sausage-shaped bacilli are some that are turbinate and taper towards one end. Early Pleistocene phosphorite nodule P895(120cm) – 1. Scale bar, 10μm. *b*, The scatter of bacterial cells in this foraminifer suggests that the bacilli may have been motile and migrated to this protected site before anchoring by the narrow end. Early Pleistocene phosphorite nodule P884(78cm) – 1. Scale bar, 10μm. *c*, The characteristic bacillary shape, now replaced by apatite, illustrates the typical morphology of the bacteria found in the East Australian phosphorite nodules. Holocene phosphorite nodule P883(20cm) – 1. The bacteria occur on a shell surface. Scale bar, 1μm.

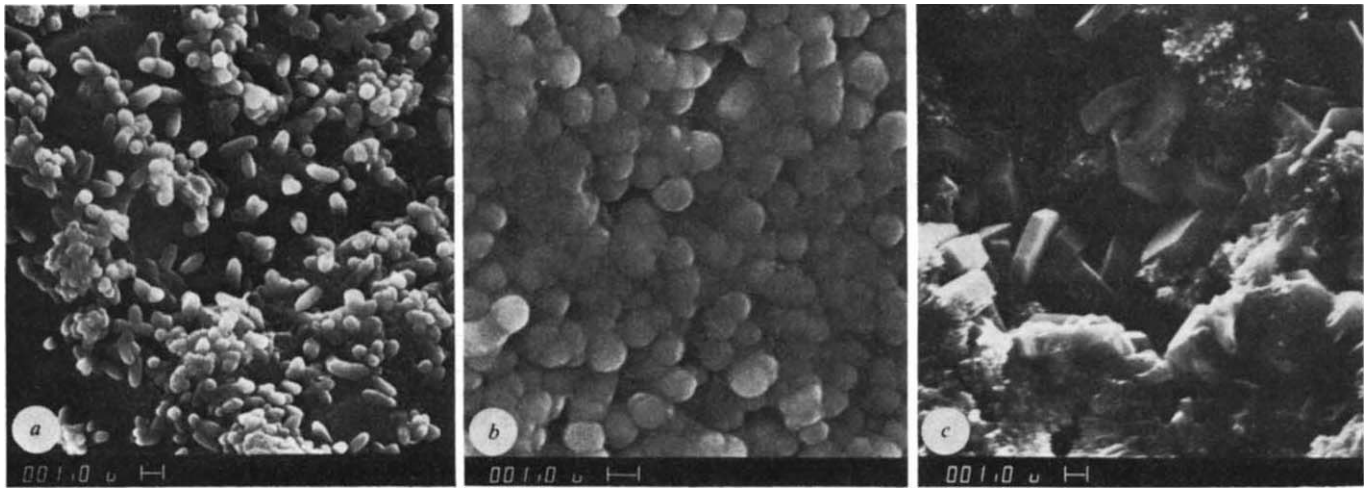

Fig. 2 *a*, In sites where bacterial growth became intensive enough to form colonies, the uniformity of cell shape and the state of their preservation are outstanding features. Holocene phosphorite nodule P883(20cm) – 1. Bacteria are clustered on detrital silicate grain. Scale bar, 1μm. *b*, In tightly-packed colonial growth the bacterial cells are polarized with long axes normal to the supporting surface, but apatite replacement still reveals confinement within cellular structures. Early Pleistocene phosphorite nodule P895(120cm) – 1. Scale bar, 1μm. *c*, Well crystallized apatite filling fracture within middle Miocene phosphorite nodule G18. Scale bar, 1μm.

tions suggests a biological desert in which few organisms grow, in which the growth rate of the individual cells may be quite slow, and in which a slow sedimentation rate provides minimal energy and/or nutrient inputs.

While there are limited criteria on which to base the identification of the bacteria, there are some morphological features which preclude membership of several taxonomic groups commonly implicated in phosphate transformations. On this basis, the sheathed bacteria which metabolize iron, the budding bacteria which metabolize manganese, and the stalked bacteria such as the caulobacters[19,20], which grow in very dilute media, are excluded. Similarly they are not slimy filamentous actinomycetes such as *Bacterionema matruchotii* or *Leptotrichia buccalis* which are found in the oral cavities of man and primates[21,22], and which deposit apatite in dental calculus and plaque deposits. The cells are not curved in the manner of spirillums or vibrios; nor flexuous, sinusoidal, nor coccal. None of the micrographs display swellings implying Gram-positive sporing rods, or the cluster formations of the corynebacteria[23].

About 90% of the bacteria isolated from marine environments are Gram-negative rods; usually pseudomonads predominate[24,25]. Many of these bacteria are not specific to marine environments, but can occur in fresh waters and soils[26]. The bacteria that we have observed are probably Gram-negative aerobic pseudomonads or facultatively anaerobic enterobacteria. The most attractive hypothesis is that they are facultatively chemolithotrophic pseudomonads with the ability to utilize hydrogen or carbon dioxide. This would have enabled them to inhabit the restricted ecological niche with very low energy input which the East Australian upper continental slope provided during phosphogenesis. A parallel group of halophobic pseudomonads inhabit reticulated distilled water storage systems where both nutrient and energy supply are drastically limiting[19].

The phosphorus contents of microorganisms are usually high, of the order of 5–10% of the organic fraction[27]. Where an organism grows very slowly and is efficient in extracting phosphorus from the low concentrations commonly present in seawater, it may accumulate normal or even elevated concentrations of nucleic acids, phospholipids and polyphosphate. The apatite forming the cell outline is not an encapsulation, but rather an intracellular deposit. It is not likely to have precipitated internally *in vivo*, but is more likely a *post mortem* transformation of assimilated phosphorus. This presupposes either that the bacteria retained cellular organization for an

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Table 1 Uranium-series isotope and age data for phosphatic nodules and calculated sedimentation rates for piston core P904 (29°18.0′ S, 153°50.3′ E, water depth, 405 m)

Depth of nodule from surface (cm)	P_2O_5 (%)	U (p.p.m.)	Th (p.p.m.)	$^{234}U/^{238}U$	$^{230}Th/^{234}U$	$^{230}Th/^{232}Th$	Age(kyr) ^{230}Th	Age(kyr) $^{230}Th_c$*	Mean sedimentation rate for stated interval (cm kyr^{-1})
35	7.9	91	3.5	1.13±0.013	0.49±0.018	43±7	<72±4	72	0–35 cm: 0.49
122	9.5	126	4.7	1.07±0.014	0.83±0.021	71±9	$<181^{+13}_{-9}$	177	35–122 cm: 0.83
146	7.2	59	5.3	1.09±0.014	0.91±0.020	33±3	>240	>240	122–146 cm: <0.38

Errors quoted based on counting statistics ($\pm 1\sigma$).
* Corrected for common Th using $^{230}Th_c = {}^{230}Th_m - (1.5 \times {}^{232}Th_{exp}(-\lambda_{230})t)$, where $^{230}Th_m$ is the measured activity of ^{230}Th.

Table 2 Site and geochemical data for east Australian continental margin phosphatic nodules and sediments

Sample no.	Description	Depth in core (cm)	Lat.	Long.	Water depth (m)	C_{org} (%)	P_2O_5 (%)	Fe_2O_3 (%)	Age (kyr)
P852		3.5–5.0	31°01.1′	153°18.7′	450	0.57	0.29	3.58	–
P895	Sediment	125	29°23.5′	153°49.3′	390	0.49	0.20	4.10	–
P906		82	22°20.8′	153°50.0′	415	0.51	0.36	5.27	–
P887	Phosphatic nodule	0–3	29°18.8′	153°50′	370	0.68	7.5	4.36	22*
G7–13		–	29°23′	153°50′	385	0.43	15.1	5.17	194*
G19		–	30°41′	153°19′	230	0.14	8.4	43.12	Middle Miocene†

* Uranium-series data. † Palaeontological data.

appreciable period after active metabolism ceased without degeneration by lysis and without leaching losses of soluble polyphosphates, or that precipitation of apatite occurred rapidly.

There is no evidence to support a hypothesis that the growth of the organism we have identified in the East Australian phosphorites needed episodic accessions of readily decomposible organic detritus. In particular, there is a lack of appreciable sulphides, an absence of fish bones in the sediments, uniformity of the bacterial population, and very low organic carbon concentrations in both the sediments and the phosphatic nodules.

While limited seasonal upwellings (and associated higher nutrient levels) are known to occur sporadically on the East Australian continental shelf[28,29], these usually occur within 15 km of the coast and are unrelated to the phosphogenic region, which is some 35–40 km offshore.

We conclude that the late Pleistocene to Holocene phosphatic nodules on the East Australian continental margin have been formed by the accumulation of carbonate fluorapatite during the *post mortem* alteration of phosphorus-rich bacterial cells. Assimilation of high phosphorus levels from seawater of average phosphorus concentration would occur slowly in a slow-growing bacterial population. For this reason a very low sedimentation rate is critical, otherwise the bacterial growth would be swamped by sediment. We envisage the microbial growth gradually cementing the unconsolidated sediments[30], although fine clay minerals in the phosphatic sediment must also be important in the lithification process. The cemented phosphatic layer is later broken into nodules, either by the action of endolithic organisms or by the effect of strong bottom currents, or probably both. The presence of fine clay within the bacteria-rich matrix suggests that bottom current velocities during times of phosphogenesis were substantially lower than present day current velocities[31,32] that are sufficiently high to winnow fines from the sediment. We are not certain of the environmental factors which controlled the onset and termination of bacterial growth and phosphogenesis, although they may have been related to a subtle balance between erosion and deposition which was controlled by variations of the East Australian current.

Carbonate fluorapatite in the middle Miocene nodules occurs largely as well-developed crystals. These may have formed during diagenesis (Fig. 2*c*) of phosphate present initially in bacterial structures. Some similarities of depositional environment of the East Australian phosphorites to other more ancient deposits of the 'East Coast' type may suggest that some of these older phosphorites also had a bacterial origin, but that this has been obscured by subsequent diagenetic processes. Structures composed of apatite and similar to those we have described also occur in phosphorites off Peru–Chile and South West Africa[6,33]. If they prove to be bacterial, our model for phosphorite formation may represent a general phenomenon.

Most samples were collected in 1979 from the vessel RV *Tangoroa* through the courtesy of Dr D. Cullen of the New Zealand Oceanographic Institute. Professor C. C. von der Borch provided samples from locations G7, G18 and G19. Dr R. C. Foster provided TEM data during early stages of the work and with Drs A. Pearce, F. S. Godfrey and B. Scott gave helpful suggestions. SEM work was carried out at the University of Adelaide Electron Optical Centre. Financial support was provided by the Australian Research Grants Committee.

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Atmospheric concentration of ammonia in Massachusetts and deposition on vegetation

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Ammonia has an important role in atmospheric chemistry and the soil nitrogen cycle, and it has long been suggested that terrestrial ecosystems may be important sources and sinks for atmospheric ammonia in both gaseous and particulate form[1–4]. But there is relatively little information on the average atmospheric concentrations of NH_3 gas and particulate NH_4^+ and their fluxes to and from ecosystems. Furthermore, much existing data are unreliable because of difficulties in separating gaseous NH_3 from particulate NH_4^+ (refs 1, 5). We have used a new method[5,6] which overcomes these difficulties and have made continuous measurements for a 1-yr period at a rural location in Massachusetts. From these data and from measurements of the rate of deposition of ammonium on plastic and natural leaves, we conclude that particulate NH_4^+ can be a significant nitrogen source for ecosystems, especially pine forests. In contrast, gaseous NH_3 is probably of little importance as its concentration is very low.

In our measurements (Fig. 1) we found a strong seasonal pattern in the atmospheric concentrations of NH_3 and NH_4^+, with maximum values during the summer months. This suggests that much of the NH_3 may come from agricultural or natural sources, rather than from the combustion of oil and coal. The measured concentrations of particulate NH_4^+ are similar to those reported elsewhere in the United States but the values for gaseous NH_3 are lower than most other reports[1,3]. The reasons for this are unknown but may be partly due to methodology. Most measurements of NH_3 have removed NH_4^+ particulates by placing a filter upstream of the NH_3 collector. Reactions on the filter may release NH_3, causing an overestimate of NH_3 concentration[1,5]. It is unlikely that the alternative methodology that we used underestimated gaseous NH_3, as placing collection tubes in series demonstrated a collection efficiency of at least 90%, which is consistent with theoretical prediction[5,6]. Another factor may be the high concentrations of acidic aerosols in the eastern United States. Calculations based on NH_4^+ and H^+ concentrations in precipitation predict[7] very low concentrations of atmospheric NH_3. Finally, there may be few major sources of atmospheric ammonia in the region, due to the acidity of the soil and low density of farms. Using similar methods, low concentrations (0.2 $\mu g\,m^{-3}$) of gaseous NH_3 have also been measured in Sweden[5], but higher values (3 $\mu g\,m^{-3}$) in the Netherlands[6].

Farquhar *et al.*[2] have determined the NH_3 compensation point, at which leaves neither gain nor lose NH_3, for several plant species. Extrapolation of their data to our mean summer temperature of 20 °C suggests a NH_3 compensation point of 1 $\mu g\,m^{-3}$, which is much higher than our mid-summer NH_3 concentration of 0.2 $\mu g\,m^{-3}$. This suggests that Massachusetts ecosystems might actually lose NH_3 to the atmosphere. Even if NH_3 uptake is possible at these low concentrations, measurements of uptake at higher concentrations suggest a deposition velocity of ~1 $cm\,s^{-1}$ (ref. 2). Assuming leaves to be present from 1 June to 1 October, this deposition velocity results in an estimated NH_3 uptake equivalent to 0.13 $kg\,N\,ha^{-1}$, which is negligible compared with the nitrogen input in precipitation.

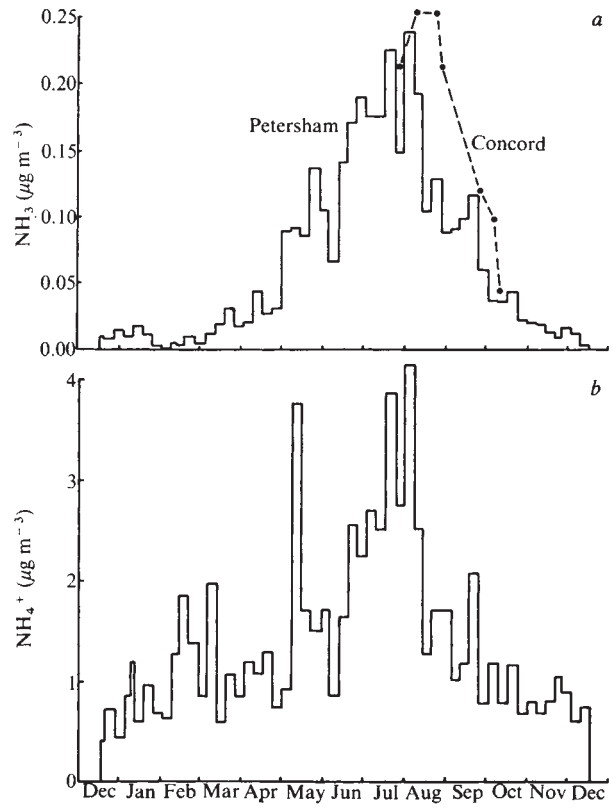

Fig. 1 Atmospheric concentrations of NH_3 (*a*) and NH_4^+ (*b*) measured during 1980 in a forest clearing at Petersham in central Massachusetts. Gaseous NH_3 was collected in glass tubing coated for 45 cm with 5% oxalic acid as described by Ferm[5]. The intake was at 1.5 m height and air was drawn through the tubing at a flow rate of 2.7 $l\,min^{-1}$, using a vacuum pump and critical orifice to control flow rate. Collections were made over 7 days with particulate NH_4^+ being collected at the downstream end of the tubing on a 0.4 μm, 47 mm, Nucleopore filter. Analysis of NH_3 was by electrode (Orion 95–10). The means of two collectors are plotted; the average difference from the mean of the individual values was 8.6% for NH_3 and 3.2% for NH_4^+. Also shown are values for NH_3 concentration (mean of two collectors) measured over 24-h periods during late summer 1980 at Thoreau's Bog, Concord, near Boston; the intake height was 0.8 m and analysis of NH_3 was by the phenol-hypochlorite method.

Unlike gaseous NH_3, particulate NH_4^+ may be a significant nitrogen source for forests in Massachusetts. However, available data do not allow an accurate estimate of the rate of deposition of particulate NH_4^+ to forests, so we made experimental determinations. A correlation coefficient of 0.67 was found between the NH_4^+ collected on rectangular plastic leaves and the concentration of particulate NH_4^+ in the atmosphere (Fig. 2). Other factors such as wind speed and particle size may also have influenced the rate of deposition, and there may have been some exchange of gaseous NH_3 between the atmosphere and the deposited aerosols. In the above experiments leaves were exposed at a height of 11 m in a clearing, but in other experiments (to be described elsewhere) there was no difference in NH_4^+ deposition on leaves placed in the canopy at two different forested sites. Likewise, there was no difference in NH_4^+ deposition on a surface area basis between natural leaves of *Kalmia latifolia* and *Pinus strobus* and plastic leaves simulating broad leaves and pine needles. Unexposed leaves were used as blanks to control for NH_4^+ arising from internal leaching. However, the rate of NH_4^+ deposition per unit of surface area was 2.0 times greater ($r = 0.93$, $P < 0.01$) for plastic leaves that simulated *Pinus strobus* needles than for rectangular plastic leaves.

Our measurements of the atmospheric concentration of particulate NH_4^+ and its rate of deposition on plastic and natural leaves can be combined to estimate the annual nitrogen input to

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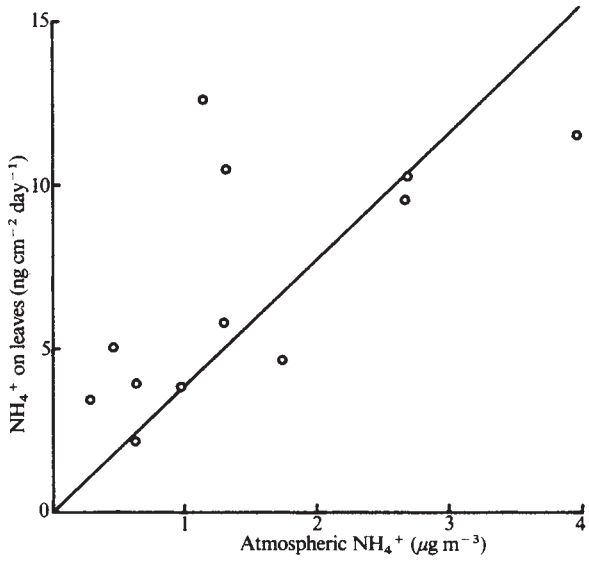

Fig. 2 Deposition of NH_4^+ on plastic leaves as a function of atmospheric concentration of particulate NH_4^+. The leaves were 10 rectangles of polyethylene (0.1 × 2.0 × 5.8 cm) that were exposed at a height of 11 m for 24 or 48 h. Ammonium was leached from the leaves by immersing each one in a vial containing 20 ml of deionized water for at least 1 min with agitation. Ammonium was measured by electrode, with a correction for unexposed blank leaves. Each point is the mean of the 10 individual determinations; the standard error averaged 12% of the mean. A linear regression through the origin is plotted: $r = 0.67$, $P < 0.02$.

forests from this source. Assuming a leaf area (both sides) of 8 m^2 per m^2 of ground surface and using the regression line in Fig. 2 we calculated a deposition velocity of 0.35 cm s^{-1} for broad-leaved trees. The calculated deposition velocity for pines was 0.72 cm s^{-1} because deposition of NH_4^+ per unit of surface area was 2.0 times greater (see above). For deciduous, broad-leaved forests the leaves were assumed to be in place from 1 June to 1 October, during which time the average particulate NH_4^+ concentration was 2.1 μg m^{-3}. For this period the calculated deposition of NH_4^+ was 0.6 kg N ha^{-1}. For pine forests the leaves were present all year and the average concentration of NH_4^+ was 1.4 μg m^{-3}. From this we calculate a NH_4^+ deposition of 2.5 kg N ha^{-1} yr^{-1}.

The above estimates suggest that dry deposition of particulate NH_4^+ on leaf surfaces may be a significant sink for atmospheric ammonia and a source of nitrogen for forests, especially pine forests. There is little nitrogen fixation in Massachusetts forests[8], so the only known nitrogen input is from precipitation which totals 6.5 kg N ha^{-1} yr^{-1} as NH_4^+ plus NO_3^- at a location 160 km to the north[9]. Thus dry deposition of particulate NH_4^+ appears to be a significant source of nitrogen relative to precipitation, while dry deposition of gaseous NH_3 is not. This supports previous evidence that dry deposition of other components of aerosols such as acids, nitrates and sulphates is important[10–14]. Further studies are needed to quantify these inputs more precisely, but it seems likely that dry deposition of aerosols containing ammonium and nitrate is an important factor in maintaining the productivity of pine and other forests. This may be especially true for forests on poor soils of low nitrogen content.

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A sterile defender morph in a polyembryonic hymenopterous parasite

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Early in the development of polyembryonic Encyrtidae, a number of larvae are formed that are morphologically different from their later sibs. These larvae die without pupating. They have been described as asexual[1], teratoid[2,3] or precocious[3], and it has been suggested that they render the host suitable as food and habitation for the later, normal larvae[1,4]. I now report findings which demonstrate that the precocious larvae constitute a defender morph, eliminating other internal parasites that would otherwise compete with, and potentially preclude the development of, their normal sibs.

A colony of an as yet undescribed species of the encrytid genus *Pentalitomastix* has been maintained in the laboratory on its natural host, the phycitid moth *Anagasta kuehniella* (Zeller). Female wasps oviposit in the host egg but the resulting parasitized host larva does not succumb until after it spins a cocoon some 6 weeks later. Instead of pupating, the host larva 'mummifies', becoming no more than a sac filled with 100–200 parasite pupae. Sequential dissections of 250 parasitized hosts reveal that as in other polyembryonic encyrtids[1,2,5], the parasite egg develops into a mass of apparently undifferentiated cells, the polygerm, which gives rise to the numerous larvae that fill up the host 'mummy'. The first one or two parasitic larvae are invariably precocious (Fig. 1) and are found in the host larva within 10 days after deposition of the parasite egg. Precocious larvae continue to appear as the polygerm increases in size (24 have been found in one host) until the stage, $4\frac{1}{2}$ weeks after egg deposition, when the polygerm dissociates into normal larvae. By the sixth week, the normal larvae are mature (Fig. 2) and only dead precocious larvae are recovered from dissected hosts.

Two solitary endoparasitic wasps, the braconid egg–larval parasite *Phanerotoma flavitestacea* Fischer, and the ichneumonid larval parasite *Trathala* sp., have also been maintained in the laboratory with *Anagasta kuehniella* as a factitious host. These parasites were originally reared from the phycitid *Ectomyelois ceratoniae* (Zeller). Dissections of 33 multiply parasitized *A. kuehniella* larvae exposed as eggs to *P. flavites-*

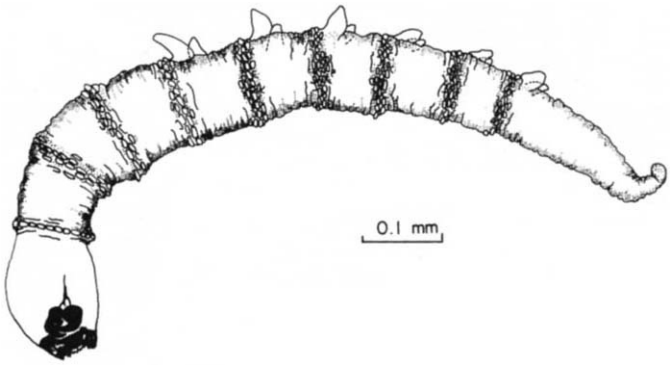

Fig. 1 Precocious larva of *Pentalitomastix* sp. recovered from 14-day-old *A. kuehniella* larva. The number of paired dorsal papillae is variable.

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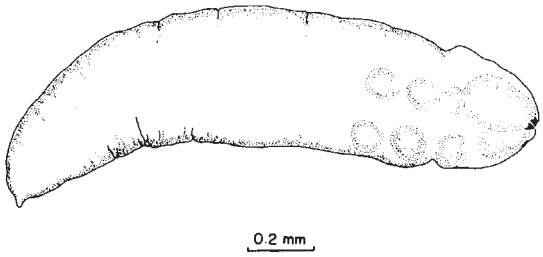

Fig. 2 One of 164 mature normal larvae of *Pentalitomastix* sp. recovered from 40-day-old *A. kuehniella* larva. Imaginal discs are apparent on the anterior third of the body.

tacea for 24 h and then to *Pentalitomastix* sp. for another 24 h each yielded one or more live precocious larvae and one wounded and dead *P. flavitestacea* larva. In 70% of these cases, the latter parasite was partially or entirely encapsulated (Fig. 3), indicating that an immune response, a common defense reaction in insects[6], had been elicited in the host larva. For the reversed sequence of parasite exposure, 64% of 50 multiply parasitized hosts yielded wounded, dead and encapsulated *P. flavitestacea* larvae.

In contrast, no wounded or encapsulated parasite larvae were found in 250 *A. kuehniella* larvae exposed as eggs to *P. flavitestacea* alone. In each of 14 observed cases of superparasitism, encounters among *P. flavitestacea* larvae occurred within 1 or 2 days of hatching of the larvae, at which time the host larva was still within its chorion. These encounters were fatal to all but one parasite larva and the losers were dismembered, not merely wounded. When 65 multiply parasitized host larvae were dissected before formation of the precocious larvae, the *P. flavitestacea* larvae were found alive. These observations, together with the extreme motility and heavily sclerotized buccal structures of the precocious larvae, indicate that they attack and kill *P. flavitestacea* larvae. This could explain why in laboratory tests, *Ectomyelois ceratoniae* (Zeller) multiply parasitized by *P. flavitestacea* Fischer and *Pentalitomastix plethoricus* Caltagirone reportedly gave rise to the latter parasite alone[7].

A. kuehniella larvae multiply parasitized by *Pentalitomastix* sp. and *Trathala* sp. yielded wounded, dead and encapsulated larvae of the latter and one or more precocious larvae of the former. Despite the similarity in appearance and motility of these larvae, the precocious larvae 'won' in all 29 cases (Fig. 4). In each of six cases of superparasitism by *Trathala* sp., all parasite larvae but one were found wounded, dead and encapsulated. In nine hosts superparasitized by *Trathala* sp. and additionally parasitized by *Pentalitomastix* sp., no *Trathala* sp. larvae survived when precocious larvae were present.

The presence of castes and intergenerational cooperation characterizes eusocial insects[8]. Although the polyembryonic

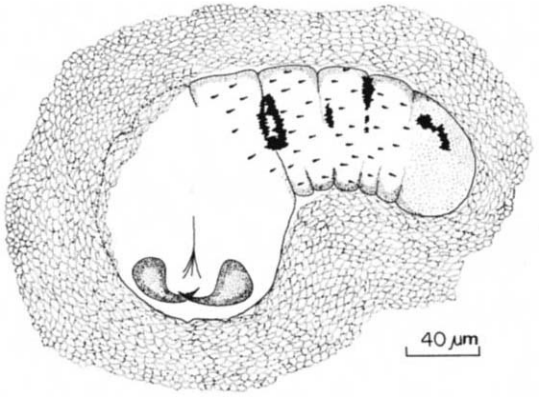

Fig. 3 Partially opened capsule containing dead, wounded, first-instar *Phanerotoma flavitestacea* larva. Wounds appear as melanized lesions on the integument. The capsule consists of layers of cells that seem to have been laid down concentrically around the dead larva.

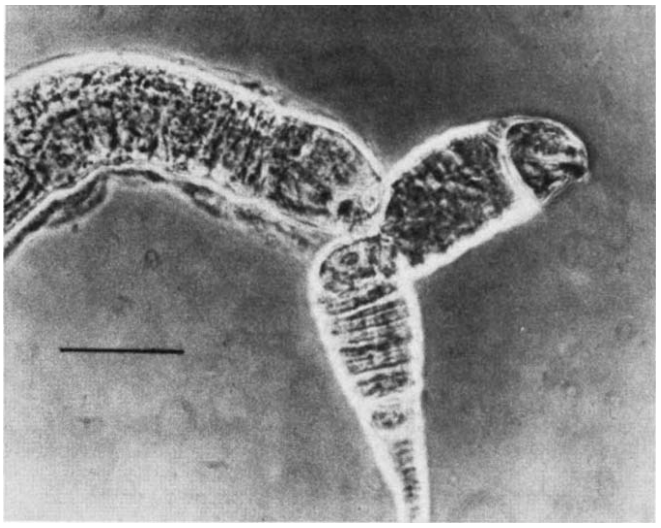

Fig. 4 Precocious larva of *Pentalitomastix* sp. (left) attacking larva of *Trathala* sp. Specimen was recovered from 49-day-old *A. kuehniella* larva exposed as an egg to *Pentalitomastix* sp. and as a 21-day-old larva to *Trathala* sp. Polaroid photograph of fresh specimen in Ringer's saline viewed through Tiyoda phase-contrast microscope. Scale bar, 0.2 mm.

Encyrtidae are not eusocial, the developmental pattern of known species involves the obligatory coexistence of a large number of individuals in a host vulnerable to attack by other internal parasites. Furthermore, any brood of parasites arising by polyembryony exists as a physically defenseless polygerm for an extended period during its own life and that of the host. Therefore, I suggest that the precocious larva serves as a defender morph and provides a means of increasing the competitive advantage and probability of survival of polyembryonic encyrtids.

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The path of axons in *Drosophila* wings in relation to compartment boundaries

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We have tested the hypothesis that the paths taken by sensory axons in the peripheral nervous system of *Drosophila* are influenced by the boundaries between developmental compartments[1]. It has been proposed that differences in cell affinity keep the epidermal cells of adjacent compartments from intermingling[2]. Such differences in cell affinity might well also prevent the axons of sensory neurones, which are derived from the same populations as epidermal cells and which use them as growth substrates, from crossing compartment boundaries. However, as we report here, we have found that sensory axons in the wing of *Drosophila* do cross the boundary between the anterior and posterior compartments of the wing.

The thoracic segments of *Drosophila melanogaster* are each divided at least into anterior and posterior (A and P) compart-

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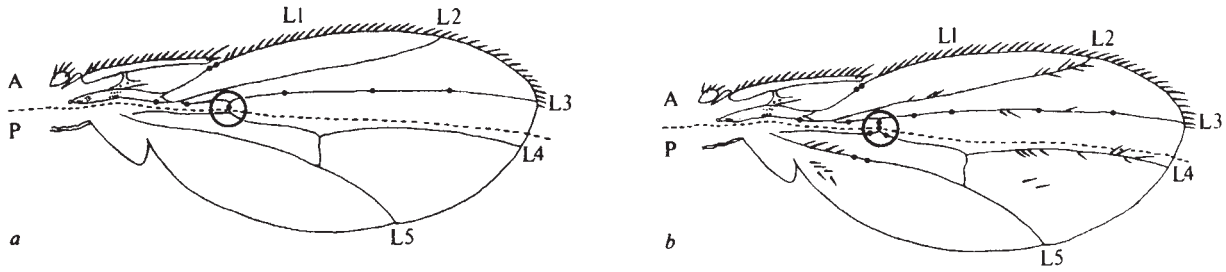

Fig. 1 Morphology of the wing of *D. melanogaster*. *a*, Wild type, with receptors on specific veins; *b*, *Hairy wing* (*Hw*), with supernumerary receptors in many locations. L1–L5, longitudinal veins 1 to 5; •, large campaniform sensilla; :::, small campaniform sensilla on the radial vein; - - - -, A–P compartment boundary; heavy circle surrounds the area of the anterior cross vein (ACV), shown enlarged in Fig. 2. The compartment boundary in *Hw* animals was determined from the behaviour of mitotic recombination clones marked with *y* and *mwh* in the progeny of two crosses: y/y; $mwh/mwh \times y\,Hw/Y$; $Dp\,sc^{J4}M(3)i^{55}/mwh^{+}$ and $y\,Hw/FM6$; $mwh/mwh \times y\,Hw/Y$; $Dp\,sc^{J4}M(3)i^{55}/mwh^{+}$. Egg collections were made over a 24-h period, and larvae aged 24–48 h were irradiated with a dose of 1,000 R (for further details, see ref. 6). Clones in flies homo-, hetero- and hemizygous for *Hw* were analysed and all behaved like similar clones in wild-type animals[5]. (For explanation of symbols and description of mutants, see ref. 16.)

ments. The presence of compartments is recognized because genetically marked clones of cells induced after a certain time in development never cross particular lines on the body surface. On the wing, the A–P boundary runs a few cells anterior to the fourth longitudinal vein[1] (L4; Fig. 1*a*).

There is evidence that compartments do influence the paths followed and the connections formed by sensory axons in the central nervous system. The particular route taken by an axon apparently depends not only on the type of receptor from which it originates, but also on the developmental compartment in which its cell body is located[3]. Furthermore, the reflex responses elicited by stimulation of similar receptors seem to depend on the compartment in which the sensory cell bodies lie[4]. Here we have investigated whether the organization of peripheral axonal pathways is also influenced by compartments.

All the known receptor cells in the wings of wild-type *Drosophila* are located in the anterior compartment (Fig. 1*a*)[5,6]. To examine the hypothesis that the A–P compartment boundary of the wing forms a barrier to axon growth, one needs receptors in the posterior compartment whose axons could potentially join the anterior nerve bundles. Flies carrying *Hairy wing* (*Hw*), a dominant, sex-linked mutation, provide suitable material for study. This mutation causes both sensory bristles and campaniform sensilla (disk-shaped cuticular strain detectors) to form in many regions of the cuticle normally devoid of receptors, including the posterior compartment of the wing. Supernumerary receptors are found on veins L2, L3, L4, L5, the anterior cross vein and occasionally on the radial vein, as well as in the inter-vein areas[7] (Fig. 1*b*).

The anterior cross vein (ACV) connects vein L4 (posterior) with vein L3 (anterior), and thus forms a potential conduit for axon growth between the two compartments. In wild-type flies this vein carries a single sensillum, located in the anterior compartment (Fig. 2*a*). Its cell body usually lies at or near the L3–ACV junction, and its dendrite travels through the ACV to attach to the dome of the sensillum. In *Hw* flies, one or more supernumerary sensilla may be present on the ACV, and extra sensilla may also occur on L4 near the ACV (Fig. 2*b–d*). In all such cases we have analysed, the axons pass through the ACV to join the nerve travelling through L3. If the cell body lies in L4 distal to the ACV (Fig. 2*c*), its axon makes a right angle turn to enter the ACV. If the cell body is located proximal to the ACV (Fig. 2*d*) its axon, in addition, first travels distally, the opposite of the usual direction. The preference of supernumerary axons in this region for following the ACV and L3 is not due to the inability of L4 to support axon growth. This is evident because the axons of distal receptors travel successfully in L4 before turning into the ACV, and receptors nearer to the proximal end of the vein commonly send their axons as a bundle through it towards the base of the wing.

Even though the nerve bundles formed by the more proximal receptors often travel towards the base of the wing, both single axons and large nerve bundles can also grow at right angles to the veins, cross the compartment boundary and join the major nerves in L3 or the radial vein (Fig. 3). The supernumerary receptors in these more proximal regions of the posterior compartment include many bristles as well as campaniform sensilla. Thus, the axons of both receptor classes readily cross the A–P boundary, and crossing is not confined to any particular location on the wing. The ACV provides an open channel, but well defined veins are not required for axons to cross into the anterior compartment.

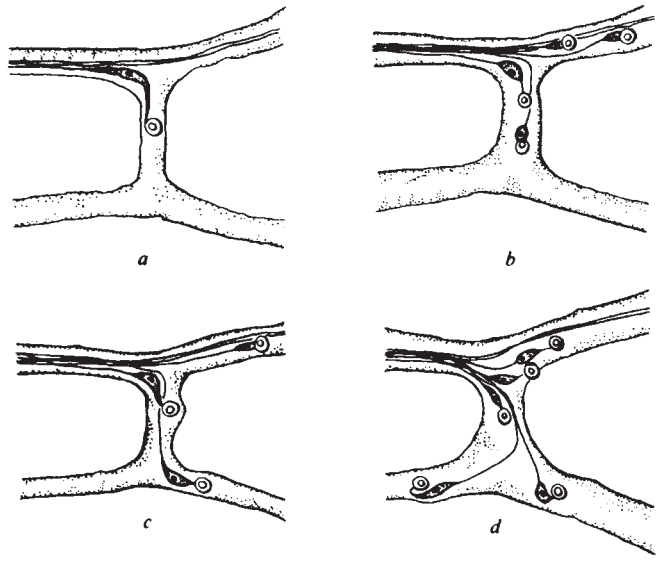

Fig. 2 Course of axons in the region of the anterior cross vein (ACV). *a*, Wild-type fly with one campaniform sensillum, its cell body at the ACV–L3 junction and its axon travelling through L3. Proximal is to the left in each drawing, distal to the right. *b–d*, Examples of homozygous *Hw* animals. Campaniform sensilla and their cell bodies are found in the posterior compartment, and their axons turn into the ACV, cross the compartment boundary and join the nerve bundle in L3. Hemizygous males and heterozygous females, which have fewer supernumerary receptors, show similar patterns. Drawn from preparations stained with methylene blue. Stain (Methylene Blue Ehrlich, Chroma, 0.05 g ml^{-1}) was injected into the thorax of adult flies and the wings were removed after 0.5–4 h and mounted in a drop of mineral oil. Contrast could often be enhanced by using differential interference optics. Over 100 wild-type and mutant flies with adequate staining were analysed.

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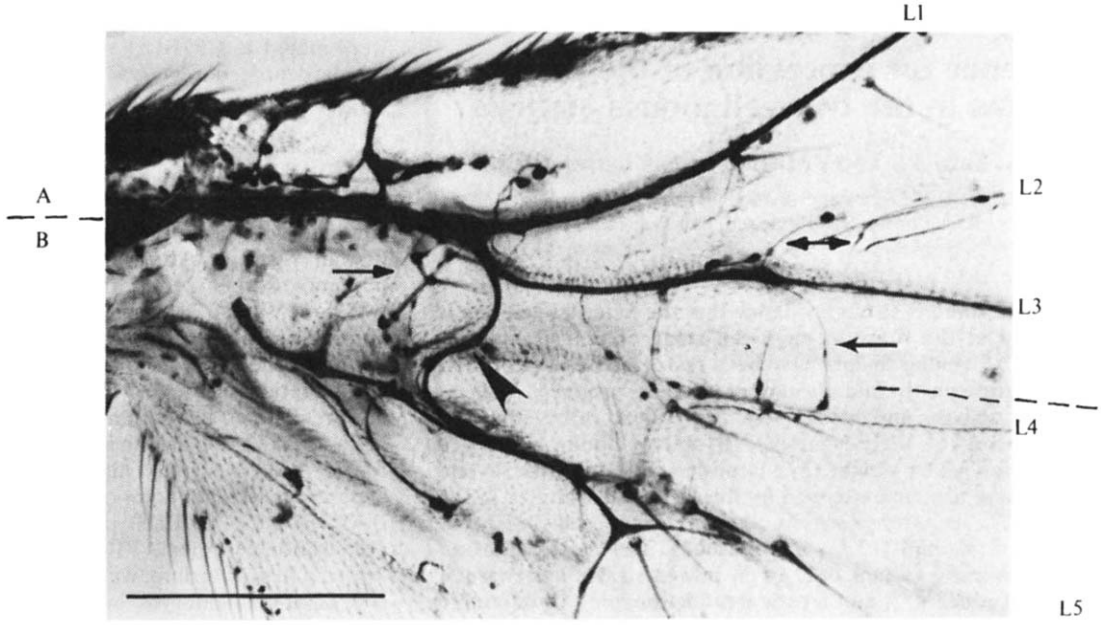

Fig. 3 Course of axons in the proximal wing of a homozygous *Hw* fly. A massive nerve bundle from the posterior compartment (◄) joins the normal anterior nerves at the confluence of veins L1 and L3. Numerous single axons cross from L4 to L3 (←), from scattered posterior receptors to the radial vein (→), and from L2 to L3 (↔). The location of the compartment boundary is indicated by the dashed lines in the margin (compare with Fig. 1). Methylene blue staining as in Fig. 2. Scale bar, 200 μm.

We have used *Hairy wing* flies to probe the potential barrier to axon growth at the A–P compartment boundary. However, it is conceivable that the *Hw* mutation, in addition to producing supernumerary receptors, might shift or eliminate the very A–P compartment boundary whose properties we want to analyse. If this were the case, sensilla which we believe to be in the posterior compartment on the basis of their location would actually be anterior in character and their axons would not be crossing a compartment boundary. To test this possibility, we induced large clones of cells marked with the mutation *multiple wing hairs* (*mwh*) in *Hw* flies (see Fig. 1 legend). These clones were confined to the same areas, and defined the compartment boundary in the same location, as in wild-type flies in all the genotypes we used for axon tracing: *Hw*/*Hw* females, *Hw*/+ females and *Hw*/*Y* males. Thus, there is no indication that *Hw* affects the compartment boundary. Furthermore, if the existence of the boundary is the outcome of affinity differences between anterior and posterior cells, these differences must also be present in *Hw* flies and must be irrelevant to the growth of adult axons.

Our clones were marked with the mutation *yellow* (*y*) in addition to *mwh*, so that the sensory bristles could also be scored. We never found *y* bristles in wild-type tissue or wild-type (y^+) bristles in a *y* clone. These observations argue strongly against the possibility that anterior bristle precursor cells have migrated into the posterior compartment.

Thus, adult axons can freely cross the A–P compartment boundary. On their way to the central nervous system they also cross at least the proximo-distal boundary[1] in the wing. On the other hand, Lawrence[8] reported that sensory axons do not cross segment borders in the abdomen of the milkweed bug *Oncopeltus*. Furthermore, he showed that clones in the epidermis are restricted early in development to an individual segment. Thus, the segment border is a compartment border on this criterion. However, the present demonstration that the A–P compartment boundary does not constitute a barrier to the growth of axons indicates that in this respect segment borders have properties which not all other compartment boundaries share.

The developmental history of axon growth in the wing is incompletely known, but the course of axons in the adult is presumably a consequence of a pattern laid down earlier. Waddington[9] reported the presence of 'nerve bundles' in veins L1 and L3 of the metamorphosing wing before the differentiation of the adult sensory cells. Preliminary studies using the electron microscope[10] indicate that these structures are much more complex than he realized, but the time of their appearance and their localization are reminiscent of the pioneer axons which have more recently been described in other insect appendages[11–13]. Experimental evidence for the causal role of such axons in the organization and placement of peripheral nerves is now available[14]. Whatever guiding factors are present in the wing, however, be they 'pupal nerves', pioneer axons, or specific non-neural substrates, we expect them to be localized primarily in veins L1 and L3. Not only are these the veins in which adult nerves are normally present, but axons from ectopic receptors often leave a particular vein at apparently arbitrary points to join the fibres travelling in L1 and L3. Figure 3 shows one example of the many we have seen of axons crossing from both L2 (an anterior vein) and L4 (a posterior vein) to join the nerve in L3.

We consider it a reasonable working hypothesis that the filopodia of growing adult axons explore the nearby territory[15], and if a suitable guiding factor is available they follow it preferentially and with the correct polarity. If the normal guiding factor is not available, other features of the environment may influence the growth of the axon but correct polarity may not be achieved (unpublished observations). In this process, compartments and the boundaries between them appear not to be important.

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Evidence for expression of the paternal genome in the two-cell mouse embryo

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Although there is strong evidence that the paternal genome is activated at the four- to eight-cell stage of mouse embryogenesis[1-12], studies to date have been restricted because of their dependence on the manifestations of gene products, such as enzyme activity and cell-surface expression, rather than on direct assay of their synthesis. β_2-microglobulin (β_2M), a 12,000-molecular weight (M_r) peptide associated with several of the gene products encoded by the *H-2-Tla* complex in the mouse[13-17], is synthesized by early cleavage stage embryos (J.A.S., T.M. and C.J.E., unpublished). The existence of an electrophoretic variant of β_2M in inbred strains has recently been described[18-20], and tryptic peptide mapping[19] and amino acid sequencing data[21] indicate that the primary sequences of these variants differ by a single amino acid, thus establishing the genetic basis for this variation. Mice heterozygous at the β_2m locus synthesize both forms, β_2M^a and β_2M^b (refs 18, 20). Using direct immunoprecipitation and two-dimensional gel electrophoresis, we have used this variation at the β_2m locus to distinguish between maternal and paternal β_2M and have now established that the synthesis of paternally derived β_2M is first detectable at the two-cell stage. This is the earliest stage at which expression of the mammalian embryonic genome has been established[22].

SWR/J superovulated female mice carrying the β_2m^a allele were mated to C57BL/6 males carrying the β_2m^b allele, and standard procedures were used to obtain fertilized eggs and preimplantation mouse embryos. Embryos from the reciprocal mating (C57BL/6♀×SWR/J♂) were also collected. The embryos were radiolabelled with ^{35}S-methionine, protein extracts were prepared and reacted with a rabbit anti-mouse β_2M serum and the antigen–antibody complexes were precipitated with protein A derived from *Staphylococcus aureus*, Cowan I strain. After release of the complexes from the immunoabsorbent, the immunoprecipitates were analysed by two-dimensional polyacrylamide gel electrophoresis and autoradiography.

Three criteria are used to verify the identity of the β_2M^a and β_2M^b peptides on the two-dimensional gels: (1) The peptides are not precipitated by preimmune rabbit serum. (2) Each peptide has a M_r of 12,000 and an isoelectric point of 6.7. The apparent M_r of β_2M^a is slightly higher than that of β_2M^b, while the apparent isoelectric point of β_2M^b is slightly higher than that of β_2M^a (Fig. 1). (3) Each peptide co-migrates with a purified preparation of either β_2M^a or β_2M^b.

Using this procedure, we tested fertilized eggs and two-, four- and eight-cell embryos heterozygous at the β_2m locus and determined that the synthesis of paternally derived β_2M is detectable as early as the two-cell stage (Fig. 2*c*). However, although β_2M synthesis is readily detectable in unfertilized eggs (unpublished), we were unable to detect the synthesis of either maternally or paternally derived β_2M in fertilized eggs (Fig. 2*a*, *b*). This finding, coupled with the observation that maternally and paternally derived β_2M are apparently synthesized in approximately equal amounts in two-, four- and eight-cell embryos (Fig. 2*c*, *d*), suggests that maternally derived β_2M messenger RNA is either degraded or otherwise inactivated at the time of fertilization and that the observed synthesis of β_2M

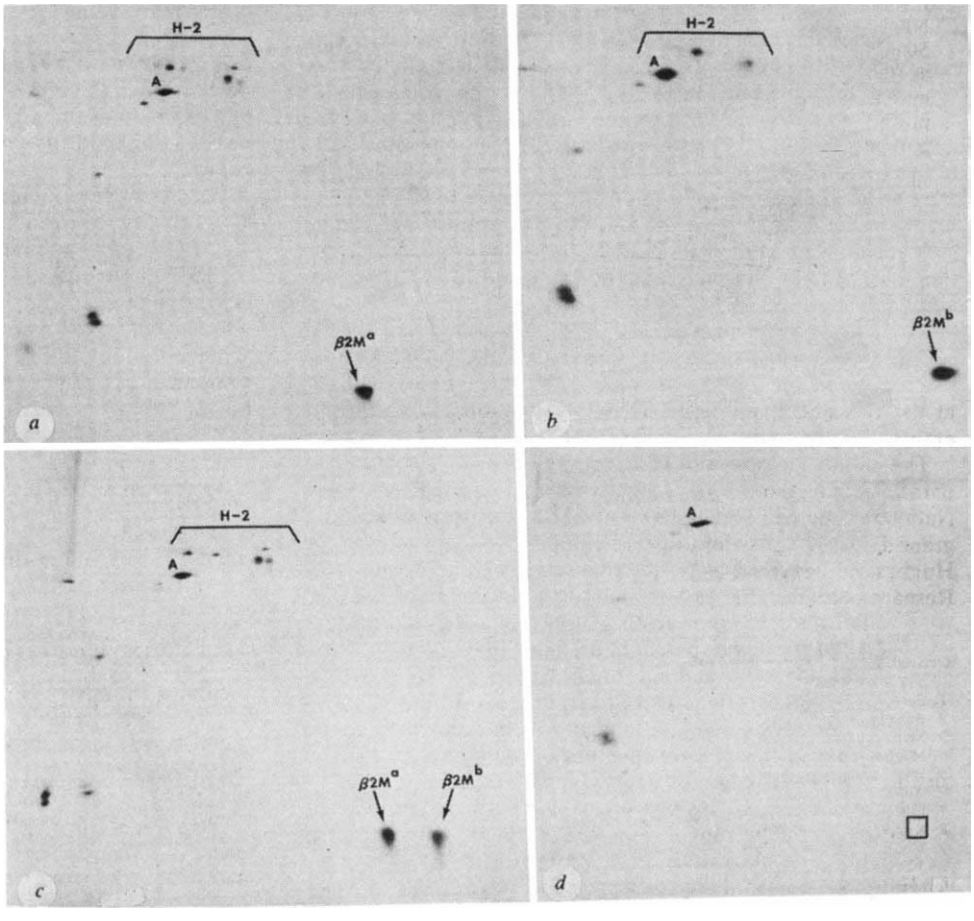

Fig. 1 Autoradiograms of immunoprecipitated polypeptides from ^{35}S-methionine-labelled splenic lymphocytes from the parental inbred strains. *a*, SWR/J lymphocytes, anti-β_2M; *b*, C57BL/6, anti-β_2M; *c*, SWR/J lymphocytes, anti-β_2M+C57BL/6 lymphocytes, anti-β_2M; *d*, SWR/J lymphocytes, preimmune serum. Radiolabelling of lymphocytes and preparation of gels were done as previously described[23]. Briefly, lymphocytes were isolated and labelled for 6 h (3×10^7 cells per ml per 250μCi ^{35}S-methionine) in Dulbecco's modified Eagle's medium lacking methionine. Aliquots of cell extracts prepared in 0.5% NP40 were reacted with antiserum for 15 min at 4 °C after which a suspension of *S. aureus* protein A (10% wt/vol) (Enzyme Center) was added and the mixture was incubated for an additional 10 min at 4 °C. Protein A-bound immune complexes were released by the addition of O'Farrell's lysis buffer[24]. The immunoprecipitates were analysed on two-dimensional gels. Non-equilibrium *p*H gradient gels having an approximate *p*H range of 4.5–7.9 were used as the first dimension[25]. Electrophoresis was carried out for 4 h at 400 V. Separation in the second dimension was done using a 4.5% polyacrylamide stacking gel and a 14% polyacrylamide separation gel. The square in *d* indicates the site to which β_2M would migrate on the gel. 'A' indicates actin, which has a M_r of 44,000. The basic side of each gel is on the right.

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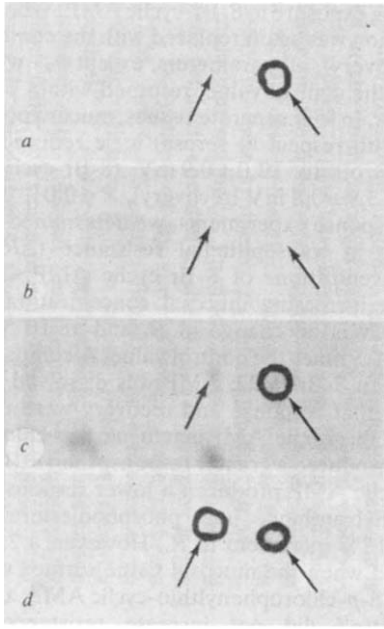

Fig. 2 ^{35}S-methionine-labelled peptides immunoprecipitated with rabbit anti-mouse β_2M serum from: *a*, fertilized eggs, SWR/J♀×C57BL/6♂; *b*, fertilized eggs, C57BL/6♀×SWR/J♂; *c*, two-cell embryos, C57BL/6♀×SWR/J♂; *d*, four-cell embryos, C57BL/6♀×SWR/J♂. The panels represent comparable regions from four two-dimensional gel autoradiograms. For each gel, 200–400 fertilized mouse eggs or preimplantation mouse embryos were obtained from superovulated females. Embryos were transferred to 30 μl of modified Whitten's medium[26,27] containing ^{35}S-methionine (specific activity 800–1,200 Ci mmol^{-1}) (Amersham/Searle) at a concentration of 2 mCi/ml^{-1} and cultured under paraffin oil at 37 °C in a 5% CO_2 atmosphere for 4 h. At the end of the incubation period, embryos were solubilized in 100 μl of 0.5% NP40 (Particle Data Inc.) in Tris-buffered saline (150 mM NaCl, 50 mM Tris, 0.02% NaN_3, *p*H 7.0). Each extract was centrifuged at 10,000*g* for 5 min in a Beckman microfuge B to remove unlysed nuclei, and the supernatants were stored at −80 °C. Immunoprecipitates were prepared as described in Fig. 1 legend. In *a* and *c*, purified mouse β_2M^b (2.75 μg) was added to each immunoprecipitate sample before electrophoresis, while in *d*, both purified β_2M^a (2.75 μg) and β_2M^b (2.75 μg) were added. Proteins were fixed and stained with 50% trichloroacetic acid–0.1% Coomassie blue, the gel was dried, and the stained β_2M spot(s) was circled with radioactive ink. The arrows in *a* and *b* indicate the absence of β_2M^a and β_2M^b, while the arrows in *c* and *d* indicate their presence. Each gel was exposed to X-ray film for $\sim 2\times10^6$ count-days (c.p.m. loaded on to gel × no. of days exposed).

at the two-cell stage is in fact the result of activation of the embryonic genome.

The rabbit anti-mouse β_2M serum and purified β_2M preparations were provided by Dr Ettore Appella from the National Cancer Institute. This work was supported by NIH grant HD-03132. C.J.E. was an investigator of the Howard Hughes Medical Institute. T.M. is a recipient of a NIH National Research Service Award.

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Regulation of epithelial tight junction permeability by cyclic AMP

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In 'leaky' epithelia, ions move through both a transcellular and a paracellular (serial alignment of tight junction and intercellular space) path. The efficiency of transepithelial transport could therefore be regulated if the cell was able to alter reversibly the permeability of tight junctions. (These are specialized regions of the apical cell membranes common to all epithelia.) We now report that such a mechanism indeed exists in the *Necturus* gallbladder. It is effected by cyclic AMP, which is already known to mediate surface membrane phenomena in a variety of cell systems through its interaction with the cytoskeletal system[1,2]. In gallbladders mounted and perfused with electrolyte solutions in an Ussing-type chamber, exposure of the mucosal surface to cyclic AMP analogues increased transepithelial electrical resistance, potential difference and short-circuit current and decreased NaCl dilution potentials in a rapid and reversible manner. We also observed rapid depolarization of cell membrane electrical potentials and a slow decline in intracellular K^+ activity. Freeze-fracture electron microscopy of tissues fixed with glutaraldehyde during the peak electrical response showed a reorientation of intramembranous junctional fibrils, suggesting that cyclic AMP reduces the ionic permeability of the paracellular pathway in this epithelium by altering the structure of tight junctions.

After decapitating the animals, gallbladders were removed, washed and mounted mucosal side up between two halves of a Plexiglass chamber. The tissue was loosely stretched over a 0.13-cm^2 chamber opening and sealed with vacuum grease between chamber halves. Tissues were bathed on both sides by a continuous flow of amphibian Ringer's solution. Transepithelial resistance (R_t), spontaneous electrical potential difference (ψ_{ms}) and short-circuit current (I_{sc}) were measured with the four-electrode technique described in Fig. 1 legend. Mucosal cell membrane electrical potentials (ψ_{mc}) were measured with a high-impedance electrometer by advancing a microelectrode (1 M KCl, >10 MΩ tip resistance) into the cells from the mucosal bath. NaCl dilution potentials were measured as the change in steady-state ψ_{ms} observed when one-half of the NaCl in the mucosal bath was replaced isosmotically with sucrose. No correction was made for liquid junction potentials because control, experimental and recovery measurements were made with the same solutions for the same gallbladder.

The gallbladders were also studied morphologically. They were fixed *in situ* by simultaneously applying a phosphate-buffered 2% glutaraldehyde solution to the mucosal and serosal surfaces. Freeze-fracture was performed at −100 °C in pre-

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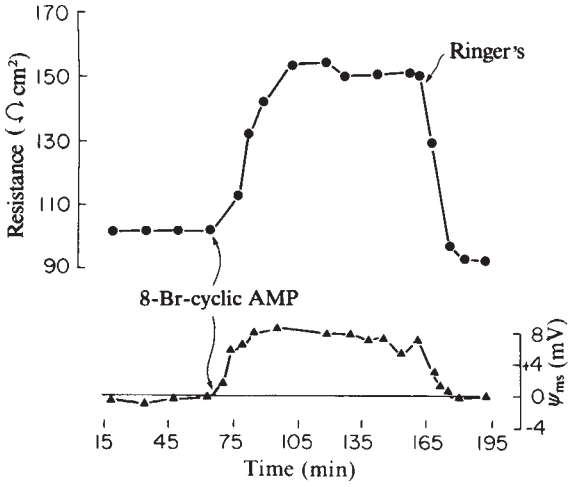

Fig. 1 Time course of a typical experiment showing a reversible increase in transepithelial electrical potential difference (ψ_{ms}, ▲) and resistance (●) on addition of 10^{-3} M 8-Br-cyclic AMP to mucosal bath of gallbladder mounted in an Ussing-type chamber. Composition of bathing solution (mM): NaCl, 87; KCl, 4.5; $CaCl_2$, 1.8; $MgCl_2$, 1.0; KH_2PO_4 0.7; $NaHCO_3$, 7.5; HEPES buffer, 5.0; *p*H 7.4, when equilibrated with 99% O_2, 1% CO_2 at 25 °C. Transepithelial electrical potential difference (ψ_{ms}, measured with respect to the mucosal bath, bottom trace), transepithelial resistance (R_t, top trace) and short-circuit current (I_{sc}, not shown in figure) were measured by an automatic voltage-clamp electrometer in which solution resistance was automatically subtracted. ψ_{ms} was measured by calomal electrodes using 1 M NaCl or 3 M KCl-filled agar bridges; R_t was measured by passing 100-μs bipolar current pulses (75 μA cm^{-2}) through the tissue via Ag/AgCl electrodes; and I_{sc} was measured by passing a current sufficient to reduce ψ_{ms} to zero.

viously fixed tissue impregnated with 20% glycerol (Denton DFE-3 apparatus) and examined with a Siemens 1A electron microscope.

Control electrophysiological parameters are summarized in Table 1 and are in general agreement with those measured by other investigators[3-5] who have also noted a low R_t in this epithelium. Figure 1 shows a typical experiment. After the baseline ψ_{ms} and R_t stabilized, the mucosal bathing solution was replaced by an identical Ringer's solution containing 10^{-3} M 8-bromo-cyclic AMP (8-Br-cyclic AMP). R_t and ψ_{ms} began to increase within 5–10 min and peaked by 30–75 min. Reversibility was indicated by a return of electrical parameters to baseline when the 8-Br-cyclic AMP mucosal bathing solution was replaced by the standard Ringer's solution. Note that, within 5–10 min, 8-Br-cyclic AMP depolarized ψ_{mc} and ψ_{cs} (the basolateral membrane potential), while R_m/R_s decreased and I_{sc} increased (Table 1). The decline in K^+ activity (a_{K^+}) was gradual during exposure to 8-Br-cyclic AMP. When the mucosal bathing solution was again replaced with the control electrolyte solution (recovery), all parameters, except a_{K^+} which rose to a value above the control value, returned within 15 min to near control values. In four separate tissues, mucosa positive dilution potentials (with respect to serosa) were reduced from 15.3± 0.4 mV (control) to 12.0±0.9 mV (8-Br-cyclic AMP) and returned to 13.9±0.8 mV (recovery), $P<0.01$.

In dose–response experiments, we determined the fractional peak change in transepithelial resistance (ΔR_t) to various mucosal concentrations of 8-Br-cyclic AMP. ΔR_t increased linearly with increasing mucosal concentrations. At 10^{-5} M ($n=3$) there was no change in R_t and at 10^{-2} M ($n=3$) R_t increased to 1.5 times the control value. A comparable ΔR_t was observed when 8-Br-cyclic AMP was dissolved in the serosal bath, except that response and recovery were delayed. On a molar basis, the cyclic AMP analogue 8-*p*-chlorophenylthio-cyclic AMP produced a greater response than 8-Br-cyclic AMP; dibutyryl cyclic AMP produced a lower response. 10^{-3} M isobutyl-1-methylxanthine, a phosphodiesterase inhibitor, produced a 12% increment in R_t. However, a 25% increment was observed when the mucosal tissue surface was pretreated with 10^{-5} M 8-*p*-chlorophenylthio-cyclic AMP, a concentration which by itself did not increase resistance. 8-*p*-chlorophenylthio-cyclic GMP (10^{-5}–10^{-4} M) reduced R_t, but the dose–response relationship was not linear and ψ_{ms} did not change.

In thin-section electron micrographs, gallbladders treated with 10^{-3} M 8-Br-cyclic AMP demonstrated thickening and aggregation of microfilaments in submembranous regions, particularly those adjacent to tight junctions. Epithelia fixed with glutaraldehyde within 5 min of exposure to 8-Br-cyclic AMP (10^{-3} M) and freeze-fractured showed basal strands in focal regions extending well below the main fibrillar meshwork. By 30 min (peak effect) this finding was more pronounced with occasional isolated strands appearing below the meshwork, resulting in an increase in number of strands and a longitudinal lengthening of the junction (Fig. 2). These morphological changes are expressed semiquantitatively by morphometric analysis (Table 2). Intercellular space morphology did not show appreciable change.

If an external current is applied across *Necturus* gallbladder, >90% of the current flows paracellularly[3,6] through the tight junction and intercellular space in series. Therefore, R_t is dominated by the ionic conductance of the paracellular pathway. Na^+ absorption in this tissue is characterized by an electroneutral NaCl-coupled mucosal influx[4] and the leaky paracellular shunt, both of which are responsible for the observed near-zero I_{sc} and ψ_{ms} during control conditions. The increase in R_t, I_{sc} and ψ_{ms} observed in 8-Br-cyclic AMP-treated tissues is consistent with reduced paracellular shunting, probably due to a reduced ionic permeability of the tight junction. A chemical or physicochemical alteration of fixed charge within the transjunctional path might decrease Na^+ or increase Cl^- conductance, causing the observed reduction in dilution potentials. On

Table 1 Electrophysiological parameters of gallbladder epithelium

	ψ_{ms}(mV)	R_t(Ωcm²)	I_{sc}(μA cm^{-2})	ψ_{mc}(mV)	R_m/R_s	ψ_{cs}(mV)	a_{K^+}(mM)
Control	−0.7±0.3	86±11	−5.4±4.3	−50±2 (30)	2.56±0.36	49±2	81±5
8-Br-cyclic AMP	6.9±0.9	127±11	64.7±16.8	−38±0 (24)	<0.10	45±1	68±2
Recovery	−0.1±0.3	82±6	−1.5±4.0	−50±2 (21)	1.24±0.20	51±2	90±7

Mean±s.e.m. from five experiments. Numbers in parentheses signify the number of successful microelectrode impalements. ψ_{cs} is basolateral membrane potential calculated from $\psi_{ms}=\psi_{mc}+\psi_{cs}$. The ratio of mucosal to basolateral membrane resistance (R_m/R_s, the voltage divider ratio) was calculated from $\Delta\psi_{mc}$ and $\Delta\psi_{ms}$, corrected for solution resistance, measured during application of 100-μs current pulses[6,10]. Intracellular K^+ activity, a_{K^+}, was measured by K^+-selective microelectrodes fabricated with Corning 47715 K^+ liquid ion exchanger by methods described[11]. Calibration plots in KCl standard solutions yielded an average slope of 56.2±2.3 mV per decade K^+ for 18 electrodes. Average values after 10^{-3} M 8-Br-cyclic AMP were recorded at peak resistance, 30–75 min after its addition to the mucosal bath. Values for the recovery period were recorded within 15 min after removal of 8-Br-cyclic AMP. Peak values are significantly different (at least $P<0.05$ by paired *t*-test) from control and recovery, except ψ_{cs} with respect to control.

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Table 2 Morphometrical analysis of tight junctions

Condition	n	No. of horizontal strands	Linear density of horizontal strands (strands per μm)	Tight junction depth (μm)
Control	102	8.0±0.2	16.1±0.5	0.56±0.02
8-Br-Cyclic AMP (5 min)	43	8.0±0.3	14.9±0.6	0.56±0.02
8-Br-cyclic AMP (peak response)	65	10.4±0.5	14.0±0.6	0.79±0.04
Recovery	60	8.9±0.3	14.8±0.5	0.62±0.02

Values are mean±s.e.m.; n is the number of grid lines drawn perpendicular to the most luminal junctional strand at 0.5-cm intervals on electron micrographs (×60,000). The average frequency of intersection of strands with grid lines determines the number of horizontal strands. Tight junction depth is the mean distance from the most luminal to most abluminal strand. 10^{-3} M 8-Br-cyclic AMP was used in all experiments. All changes measured at peak response were significantly different from control and recovery (at least $P<0.05$ by unpaired t-test), except for the change in linear density with respect to recovery. Cyclic AMP induces a reversible longitudinal expansion of the fibrillar meshwork.

the other hand, reduction of dilution potentials by cyclic AMP may simply reflect a change in dimensions of a normally cation-selective channel.

Cyclic AMP has additional effects on cell membrane transport in this epithelium. 8-Br-cyclic AMP may increase Na^+ conductance of the mucosal membrane, as demonstrated by the depolarization of ψ_{mc} and decreased ratio of mucosal to basolateral membrane resistance. Assuming no change in R_s, we calculate that mucosal membrane conductance increased 25-fold. This is consistent with our observation that $\Delta\psi_{mc}$ virtually disappeared after exposure of the mucosal surface to 8-Br-cyclic AMP. Depolarization of ψ_{mc} (and ψ_{cs}) and an increase in ψ_{ms} with increased mucosa to serosa Na flux (and I_{sc}) have been observed in *Necturus* gallbladder exposed to the antibiotic amphotericin B, a drug thought to increase mucosal membrane conductance to cations[4,5]. However, increased mucosal membrane conductance cannot account for the observed decrease in NaCl dilution potentials or increased R_t, which require an independent effect on tight junction conductance. An electrical circuit model of *Necturus* gallbladder epithelium predicts that an increase in the shunt pathway resistance alone will depolarize ψ_{mc}[3]. However, this model also predicts a hyperpolarization of the ψ_{cs} in these conditions rather than the 4-mV depolarization observed here. The gradual decrease in a_{K^+} after mucosal exposure to cyclic AMP is consistent with a change in cell membrane permeability to K^+ or may result from the observed cell membrane depolarization. Thus, intracellular electrolyte shifts and/or changes in cell membrane permeability may account for our failure to observe the predicted hyperpolarization of ψ_{cs}.

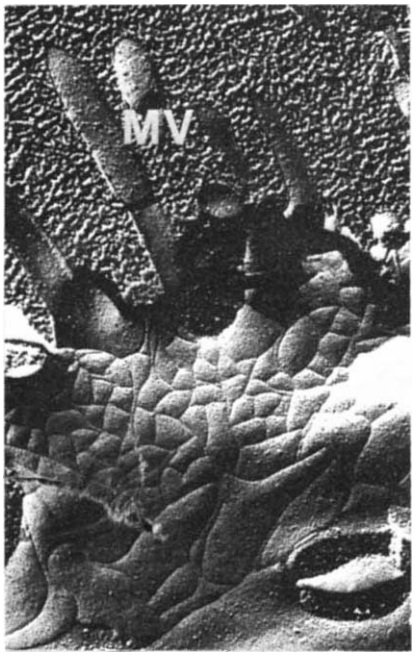

Fig. 2 Freeze-fracture of gallbladder epithelium fixed at peak response (10^{-3} M 8-Br-cyclic AMP). Note the increased number of grooves below the meshwork. Microvilli (MV) are labelled (×45,000). In control gallbladders fixed while bathed in amphibian Ringer's solution, strands form a regular meshwork. Occasionally an abluminal strand will extend down from this meshwork (see ref. 7).

The exact mechanism of cyclic AMP modulation of tight junctional permeability is not defined. Plant cytokinins behave electrophysiologically like a phosphodiesterase inhibitor while altering microfilament morphology[7]. As cyclic AMP reacts with the cytoskeleton in other cell systems, it may have a specific role in the orientation of tight junctional intramembranous strands, thereby regulating paracellular ion flow. Uncertainties as to the nature of the transjunctional ionic pathway restrict speculation on structure–function correlations. However, in a simple conductor, resistance is directly proportional to length and in these experiments resistance increased ~50% while junctional depth increased ~40%. Cyclic AMP may not affect all leaky epithelia in the same way; indeed, in mammalian kidney proximal tubule it increases transepithelial ^{14}C-sucrose flux[8], while in rabbit ileum it increases transepithelial electrical resistance[9].

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Increase in faecal nitrosamines in Japanese individuals given a Western diet

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Colon cancer is prevalent in North America and Western Europe, but has a low incidence in Japan[1,2]. Epidemiology suggests that a high intake of animal fat[3,4] and protein[5,6], especially beef[7,8], is associated with colon carcinogenesis. Numerous hypotheses have been proposed to account for the relationship between these diets[9–13], but experimental support for them is unconvincing. The specific carcinogen involved in cancer of the large bowel remains to be identified experimentally. Bruce and co-workers[14] showed that several volatile nitrosamines were present in normal human faeces and suggested that these were produced endogenously in the lower intestine but they subsequently negated this hypothesis[15]. We demonstrate here that the levels of volatile nitrosamines in human faeces are markedly increased in Japanese individuals given a Western-style diet, but decreased by a typical Japanese diet.

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We studied six healthy Japanese male volunteers from our laboratory (aged 27–39 yr) who were not receiving medical treatment during this study. Three of the subjects were given, in order: (1) a typical Japanese diet for 4 days; (2) a mixed Japanese diet for 3 days; (3) a balanced Western-style diet for 8 days and then a typical Japanese diet for 6 days. Four of the subjects were given, in order, a long-term mixed Japanese diet followed by a high-fat, high-meat diet for 4 days. These diets are shown in Table 1. The typical Japanese diet consists of boiled rice and side dishes of fish and vegetables. This diet provided ~2.0 kcal, 15% protein (mostly cereal protein), 20% fat, 65% carbohydrate and 75 mg vitamin C. In the case of the balanced Western-style diet, the staple food is meat, with side dishes of bread and vegetables. For breakfast, orange juice was replaced by vegetables to provide nitrate intake. This diet provided ~2.8 kcal, 25% protein (mostly animal protein) 55% fat, 20% carbohydrate and 70 mg vitamin C. The mixed Japanese diet consists of various food items, including those typical of Western, Chinese and Japanese diets, and is popular among contemporary Japanese. This diet provided ~2.4 kcal, 20% protein, 30% fat, 50% carbohydrate and 90 mg vitamin C. The high-fat, high-meat diet is mostly animal fat and meat with small amounts of bread and vegetables, which provides ~3.0 kcal, 30% protein, 65% fat, 5% carbohydrate and 15 mg vitamin C. Nitrate contents of these diets were estimated according to Walker[16] and Yanagihara *et al.*[17] to be: typical Japanese diet, 450 mg; balanced Western-style diet, 360 mg; mixed Japanese diet, 250 mg; and high-fat, high-meat diet, 50 mg.

Table 1 Composition of diets

	Food item	Amount
Typical Japanese diet		
Breakfast	Rice, boiled	1 cup
	Soup, containing bean paste (*miso*) and bean curd (*tofu*), vegetable or seaweed	1 cup
	Egg, raw or cooked	1
	Seaweed (dried laver (*nori*)) and/or vegetable	5 pieces
	(Chinese cabbage, spinach, etc.), boiled	50 g
	Pickled vegetable	Little
	Japanese green tea	1/2 cup
Lunch	Rice, boiled, or noodles	2 cups
	Soup (similar to breakfast)	1 cup
	Fish, broiled	70 g
	Vegetable (same as breakfast)	50 g
	Pickled vegetable	Little
	Fruit (Japanese mandarin)	1
	Japanese green tea	1 cup
Dinner	Rice, boiled	2 cups
	Soup (similar to breakfast)	1 cup
	Fish, broiled, boiled, fried or raw	70 g
	Cooked vegetables (4–5 kinds of raddish, carrot, burdock, onion, potato and mushroom (*shiitake*), added fish paste or chicken)	1 cup
	Pickled vegetable	Little
	Fruit (apple or Japanese mandarin)	1
	Japanese green tea	1/2 cup
Balanced Western-style diet		
Breakfast	Egg and bacon or ham, fried	150 g
	Bread and butter	1 slice
	Vegetables (1–2 kinds of lettuce, celery and spinach), salad or fried	100 g
	Whole milk	1 cup
	Coffee	1/2 cup
Lunch	Beef, broiled	250 g
	Bread and butter	1 slice
	Vegetables (2–3 kinds of lettuce, celery, spinach, potato and carrot), salad or fried	150 g
	Soup, containing milk and corn	1 cup
	Fruit (banana)	1
	Coffee or black tea	1 cup
Dinner	Beef, broiled or stewed	250 g
	Bread and butter	1 slice
	Vegetables (similar to lunch)	150 g
	Soup (similar to lunch)	1 cup
	Fruit (apple or banana)	1
	Ice cream or cake	1
	Soft drink	1 cup
	Coffee	1/2 cup
High-fat, high-meat diet		
Breakfast	Eggs and bacon or ham, fried	200 g
	Bread and butter	1/2 slice
	Vegetable (lettuce or cucumber), salad	Little
	Coffee	1/2 cup
Lunch	Beef, broiled	330 g
	Bread and butter	1/2 slice
	Vegetables (potato, carrot and/or onion), salad or fried	Little
	Coffee or black tea	1 cup
Dinner	Beef, broiled	350 g
	Bread and butter	1/2 slice
	Vegetables (similar to lunch)	Little
	Soup, containing milk and corn	1 cup
	Coffee	1/2 cup
Mixed Japanese diet		
Variety of items from Western, Chinese and typical Japanese diets		

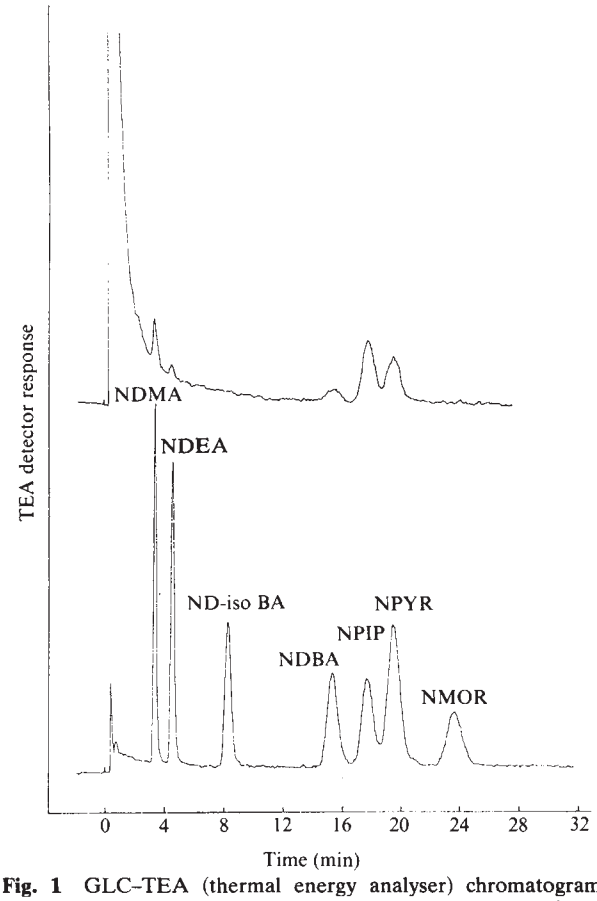

Fig. 1 GLC–TEA (thermal energy analyser) chromatograms obtained for 10 μl dichloromethane extracts of human faeces (upper part) and a standard mixture of volatile nitrosamines (lower part). Fresh faeces were immediately homogenized and weighed: 5 g faecal samples were homogenized in a Teflon homogenizer with 8 g sodium chloride and 20 ml 2 M dosium hydroxide. The samples were then transferred to two 50-ml centrifugation tubes having ground stoppers, and extracted three times with 30 ml dichloromethane (absorbancy analysis reagent; Dōjin) using a shaker. The dichloromethane was separated by centrifugation at 3,000 r.p.m. for 10 min. The extracts were washed with a small volume of distilled water in a separating funnel, and dried over anhydrous sodium sulphate. Sodium sulphate was then washed three times with a small volume of dichloromethane. The combined extracts were evaporated to 0.5 ml in a Kuderna–Danish concentrator at 33 °C for ~1 h. A gas-liquid chromatograph connected to a thermal energy analyser (model TEA-502, Thermo Electron) was used to detect nitrosamines. Glass tubing (2 m × 3 mm) packed with 25% PEG 6,000 on chromosorb W, 60–80 mesh was used in a single-column isothermal gas chromatograph. The carrier gas was argon (flow rate 50 ml min^{-1}) and the column temperature was 160 °C. TEA attenuation was ×4. The recovery rates of NDMA, NDEA, NDBA, NPIP, NPYR and *N*-nitrosomorpholine (NMOR) (added to the faeces at a concentration of 1 mg per kg) were 69, 75, 78, 81, 82 and 81%, respectively. The detection limit was in the range 0.2–0.5 μg per kg.

Several volatile nitrosamines were detected in normal human faeces (Fig. 1). The five peaks were identified as *N*-nitroso-

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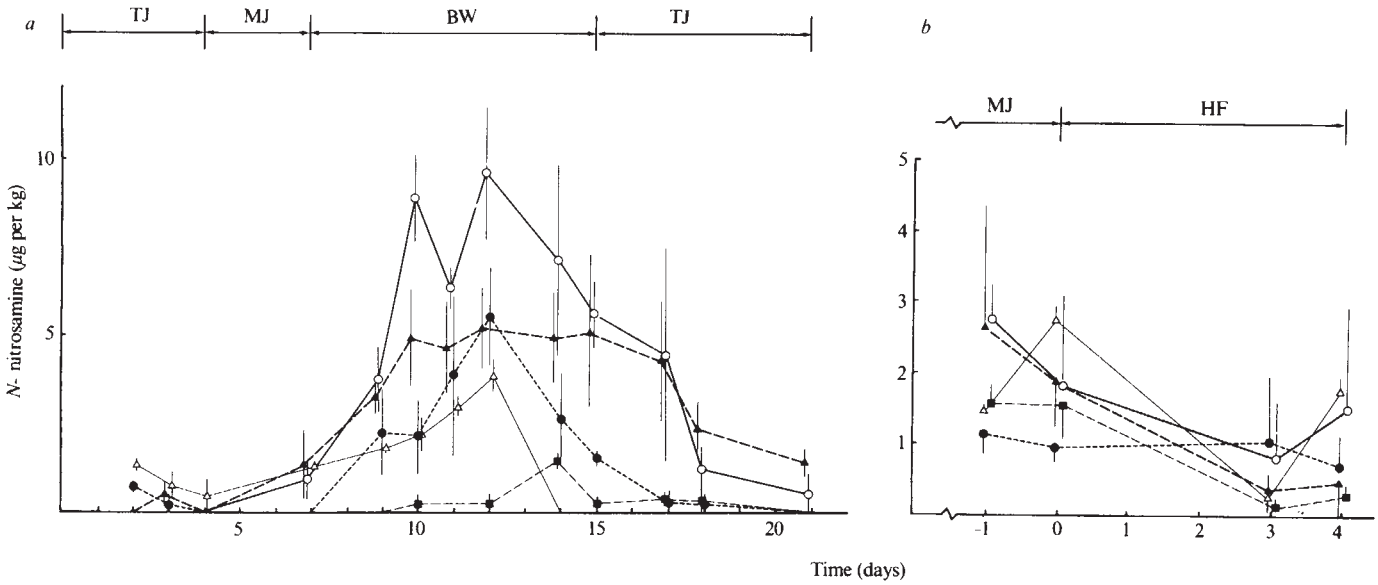

Fig. 2 Changes in levels of volatile nitrosamines in human faeces from individuals on Japanese and Western-style diets (*a*), and mixed Japanese (MJ) and high-fat, high-meat (HF) diets (*b*). ●, NDMA; ■, NDEA; △, NDBA; ○, NPIP; ▲, NPYR. TJ, typical Japanese diet; BW, balanced Western-style diet. Values represent the mean ± s.e.m. for three or four subjects.

dimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR) by comparison with standards. A control experiment showed that the reagents and glassware used in the analysis did not contaminate the volatile nitrosamines, and that the addition of 1 M sulphamic acid (which decomposes NO_2^-), 50 mM ascorbic acid (which inhibits nitrosation of amines[18]) or 0.1 M sodium nitrite (a precursor of nitrosamines) to the faecal samples did not affect the yield of nitrosamines. Furthermore, we analysed the faecal samples immediately after collecting specimens. These experiments indicated that there was no artificial formation of nitrosamines during either the analysis[15] or storage[19]. To extract volatile nitrosamines from faeces using dichloromethane, the distribution ratio must be improved by salting out and alkalization of the faecal samples. Volatile nitrosamines in faeces may exist in a state which is difficult to extract, possibly because the nitrosamines are contained in undigested food, or bound chemically or physically to some compounds.

The changes in levels of volatile nitrosamines in faeces observed for the different diets are shown in Fig. 2. Each subject showed a similar pattern of nitrosamine levels. In the case of the typical Japanese diet, faeces contained NDMA, NDBA and NPYR at levels below 1.7 μg per kg. The amount of faecal nitrosamines detected varied from day to day. After 3 days on the mixed Japanese diet, NPIP appeared and the concentration of NPYR increased. In the case of the balanced Western-style diet, these nitrosamines increased in faeces markedly, particularly NPIP, which reached a maximum of 13.3 μg per kg. The maximum total amount of nitrosamines was 26.6 μg per kg. NDMA, NPIP and NPYR were detected constantly during the study period for the balanced Western-style diet, but no NDBA was detected on the latter days of the diet. After the change from the balanced Western-style diet to the typical Japanese diet, the levels of these nitrosamines in faeces were reduced but not rapidly. NDEA was detected at a maximum of only 1.8 μg per kg during this experimental period, and there was no clear change in amounts of NDEA with change in diet. On the other hand, these nitrosamines decreased in individuals when taken off the mixed Japanese diet and given the high-fat, high-meat diet (Fig. 2*b*).

These results suggest that volatile nitrosamines in faeces do not originate from nitrosamines formed in fat during cooking[20,21], but are formed in the intestine from precursors contained both in meat and vegetables. *N*-nitrosamines may be formed from precursors in the intestine in the following way. Nitrate, which is largely contained in vegetables[16,17], is reduced to nitrite by oral or intestinal bacteria[22,23]. Amino acids and lipids, largely contained in meat, are decomposed to secondary amines by the intestinal bacteria[24,25]. Finally, *N*-nitrosamines are formed from nitrite and secondary amines by the intestinal bacteria in the neutral or weak alkaline conditions of, for example, the lower intestine[26,27]. It is considered that the low levels of volatile nitrosamines found in faeces of individuals given the typical Japanese diet or the high-fat, high-meat diet are due to the low amounts of amino acids and lipids in the former diet and the low amounts of nitrate in latter. Sufficient quantities of nitrate and amino acid or lipid, such as in the balanced Western-style diet, will be necessary for the formation of nitrosamines in the intestine. It is known that vitamin C inhibits nitrosation of amines[18]. The vitamin C content in the balanced Western-style diet was nutritionally sufficient, but was insufficient to inhibit nitrosation. The average level of total volatile nitrosamines in faeces in the case of the nutritionally balanced Western-style diet was 10 times that in faeces from those given the typical Japanese diet. This may have strong implications for the aetiology of colon cancer. It is possible that *N*-nitrosamines in the faeces are responsible for many other kinds of cancer, for example, breast and prostate, as these show the same epidemiological pattern as colon cancer[1,2]. Further investigation of the carcinogenicity of *N*-nitrosamines, and the inhibition of *N*-nitrosamine formation in the intestine by the control of diets or intestinal bacteria is needed.

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Anti-PGE antibodies inhibit *in vivo* development of cell-mediated immunity

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Prostaglandins have been implicated in the regulation of humoral and cell-mediated (CMI) immunity, but little is known about their precise mode of action; published data indicate both enhancing and suppressing activity of these substances[1–4]. E-type prostaglandins (PGEs) act as extracellular modulators of the function of T lymphocytes[5,6] and there may be differences in the sensitivity of different T-cell subpopulations to this modulation[7–9]. As most studies have used *in vitro* test systems, it is unknown whether prostaglandins have the same effects *in vivo*[4]. We have therefore examined the role of PGEs in CMI *in vivo*. Prostaglandins are derived from essential fatty acids (EFA) via the cyclooxygenase system, and inhibition of this pathway by aspirin or indomethacin[10,11] has been widely used to study prostaglandin effects. However, the usefulness of these substances *in vivo* is restricted by their toxicity and limitations in their selective action[12,13]. An alternative is to use anti-prostaglandin antibodies, which antagonize F- and E-type prostaglandins in experimental animals[14,15] and prevent PGI_2 from inhibiting ADP-induced platelet aggregation[16]. We report here that anti-PGE antisera significantly suppressed experimental allergic encephalomyelitis (EAE) in rats and prevented the generation of host-versus-graft (HvG) and graft-versus-host (GvH) reactions in mice, suggesting that PGEs are important mediators not only of *in vitro* induction of CMI responses[8,9] but also of the early phase of CMI *in vivo*.

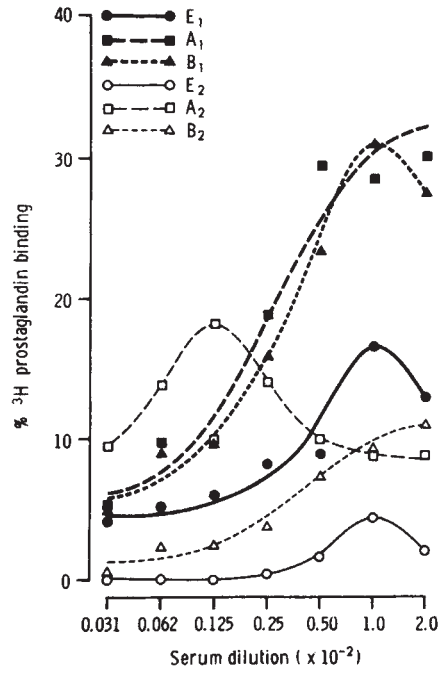

Fig. 1 Radioimmunoassay of an anti-PGE_1 antiserum (APS) raised in Lewis rats by s.c. injection of 0.2 mg of a PGE_1–BSA conjugate[17,18] emulsified in complete Freund's adjuvant (CFA) followed by two injections, at monthly intervals, of 0.1 mg of conjugate in CFA. Serum was collected on day 10 after the third inoculation of antigen and thereafter at monthly intervals, 10–12 days after further boosting with 0.1 mg of conjugate. A similar protocol was used for the production of APS in rabbits and mice with the doses of antigen adjusted to the different body weights: rabbits received 2.0 and 1.0 mg and mice 0.02 and 0.01 mg of the conjugate. Control sera were raised by the same method but using injections of BSA in CFA only. Anti-PG activity was tested by radioimmunoassay. Serial dilutions of APS were carried out in V-shaped microtitre plates (Sterilin). ^{3}H-labelled prostaglandins E_1, A_1, B_1 and E_2, A_2 and B_2 (specific activity 50 Ci mmol^{-1}; Amersham) were added to the wells at an activity of ~12,000 counts per min. After incubation for 1 h at 37 °C, 20 μl of anti-rat IgG (H+L; Nordic Immunology Laboratories, UK) and 15 μl of normal rat serum (diluted 1:100) were added. After further incubation for 16 h at 4 °C the plates were centrifuged (500*g* at 4 °C for 30 min). The supernatants were decanted and the precipitates dissolved in 0.2 ml of 0.1M NaOH and transferred to 2 ml NE260 scintillation fluid (Nuclear Enterprises). Radioactivity was measured in a liquid scintillation counter. All tests were carried out in triplicate. A background activity of 5–6% was measured in the control serum as well as in normal rat serum, and this activity was subtracted from that found for APS.

Anti-prostaglandin antiserum (APS) was raised by repeated subcutaneous (s.c.) injections in Lewis rats, NZW rabbits or CBA mice of a conjugate of PGE_1 and bovine serum albumin (BSA) emulsified in complete Freund's adjuvant[17,18]. Anti-prostaglandin activity of these sera compared well with those reported elsewhere[16–19]. Figure 1 shows the anti-PGE_1 activity

Table 1 Suppression of EAE in Lewis rats by antisera containing antibodies to PGEs

Expt	Group	No. of rats per group	Treatment	EAE prevalence on various days after antigen inoculation: Day 13	Day 15	Day 17	Day 19	Day 21	Day 23	Day 29
1	*a*	6	Control serum	0 (0)	2 (3)	6 (20)	6 (17)	6 (17)	6 (18)	6 (17)
	b	6	APS	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	2 (8)	2 (8)
2	*a*	8	Control serum	2 (2)	5 (7)	7 (20)	8 (34)	8 (30)	6 (22)	4 (8)
	b	8	APS	0 (0)	0 (0)	0 (0)	1 (1)	1 (6)	1 (6)	2 (8)
Difference in EAE prevalence between groups *a* and *b**				NS	$P \leq 0.01$	$P<0.002$	$P<0.002$	$P<0.002$	$P \leq 0.002$	NS

EAE was produced by s.c. injection of guinea pig brain stem and spinal cord material emulsified in complete Freund's adjuvant[21,22], and severity of clinical disease scored in the range 0 (= no disease) to 8 (= death due to EAE)[21,22]. The batches of control serum and APS used were raised in Lewis rats (expt 1) or NZW rabbits (expt 2). APS or control serum (0.25–0.30 ml) was injected three times weekly between day 0 and day 21 (expt 1) or day 23 (expt 2). After discontinuation of the treatment, a few more animals in the two APS groups developed EAE, but no further increase in disease prevalence was observed after day 29 (for a further observation period of ~2 months). EAE prevalence = no. of rats per group showing clinical signs of EAE at any one of the days indicated; the sum of clinical scores per group is given in parentheses.

* Significance by Fisher's exact test for experiments 1 and 2 pooled. NS, not sigificant.

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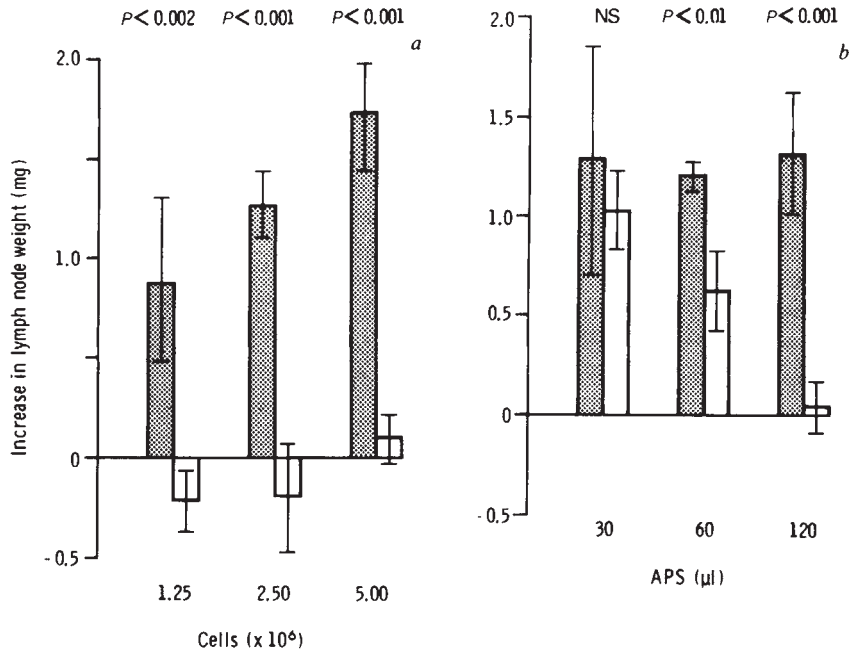

Fig. 2 The effect of APS on HvG reaction in CBA mice. CBA mice were inoculated in the left footpad with irradiated (2,000 rad) spleen cells of A strain mice, and the right footpad was injected with irradiated spleen cells from CBA mice. The extent of the resulting reactions was determined by measuring the increase in weight of the left popliteal lymph node over the weight of the right node. *a*, The weights of the popliteal lymph nodes were measured on day 3 after inoculation of the mice with various concentrations of irradiated spleen cells. APS or control serum (derived from CBA mice) was injected s.c. into the nuchal region at a dose of 30 µl per day. The first injection was on day 0, immediately after inoculation of the cells (time 0), and was followed by further injections on days 1 and 2. *b*, APS or control serum (derived from CBA mice) was given as a single s.c. injection on day 0 immediately after footpad inoculation of CBA mice with a fixed number (5×10^6) of spleen cells. Columns represent the mean increase in weight (±s.d.) of the left popliteal lymph nodes over the weight of the right popliteal nodes in groups of six mice. Shaded and open columns represent treatment with control serum and APS, respectively. The differences between control groups and APS-treated animals were assessed using Student's *t*-test. NS, not significant.

of a batch of APS used to treat EAE. Dehydration and rearrangement of double bonds can result in the conversion of some of the PGE_1 into PGA_1 and PGB_1 during the conjugation of PGE_1 to BSA. A similar conversion may also occur after injection of the conjugate, due to the action of the recipients' dehydrating and isomerizing enzymes[20]. Thus, not only did we detect anti-PGE_1 activity but also activities against PGA_1 and PGB_1, as well as cross-reactivity with PGE_2 and its respective derivatives (Fig. 1). As we were less successful in raising APS by injection of a PGE_2–BSA conjugate , all experiments reported here used only serum produced by injections of PGE_1–BSA. Control sera were raised by injection of BSA emulsified in complete Freund's adjuvant, using the same method as for production of anti-PG antiserum.

We first tested the effect of APS on EAE. This CMI disease was induced in Lewis rats and its activity determined as described previously[21,22]. Treatment with s.c. injections of APS from the day of antigen inoculation (day 0) up to days 21–23 suppressed EAE (see Table 1). Not only were there significantly fewer animals developing clinical signs of EAE but also onset of the disease was delayed in the APS-treated groups.

As the use of EAE in rats was too complicated for investigating the dose–response effect of APS and its inhibitory action in relation to the time of administration, we used mice in further experiments. HvG and GvH reactions induced by injection of allogeneic cells or cells of parental strain animals were measured in a lymph node assay system. We found that APS treatment significantly suppressed both HvG and GvH reactions in mice. Injections s.c. of 30 µl APS per day, from day 0 to day 2 after challenge of CBA mice with A strain spleen cells almost completely inhibited the HvG reaction (Fig. 2*a*). The inhibitory effect of a single dose of APS given immediately after the injection of donor cells was dose dependent in HvG (Fig. 2*b*) as well as in GvH experiments (Fig. 3). GvH reactions were reduced to about half by 20–30 µl APS; 60 µl were needed to achieve a similar effect in HvG reactions. Studies of the effect of APS in relation to time of its administration showed that the sera were most effective during the first hours after challenge of the animals with donor cells (Fig. 4). This was most pronounced in HvG reactions: the inhibitory effect of a single dose of APS (120 µl) given at time 0 was much greater than the effect of APS given 6 h after the injection of donor cells. In GvH reactions an inhibitory effect was still observed when APS was administered as late as 24 h after cell injection. However, inhibition was again greatest during the first hours after the inoculation of parental strain cells.

PGEs have been found to inhibit mitogen-induced lymphocyte proliferation[23–25], lymphocyte-mediated cytolysis[26] and the production of lymphokines[5,6,27]. Treatment *in vivo* with PGE_1 prevented development of anaemia and progression of nephritis in murine lupus erythematosus[28,29] and the development of hypersensitivity responses was reduced in mice[30]. Furthermore, PGE_2 restricted lymphocyte traffic through antigen-stimulated lymph nodes, possibly by modulating the interaction of the lymphocytes with adherent cells within the nodes[31]. In two recent studies *in vitro*[8,9], PGEs affected the induction phase of one-way mixed lymphocyte reactions and development of lymphocyte cytotoxicity. Cell proliferation as well as cytotoxicity were enhanced when the cultures were treated with indomethacin, but the response was restored to normal by

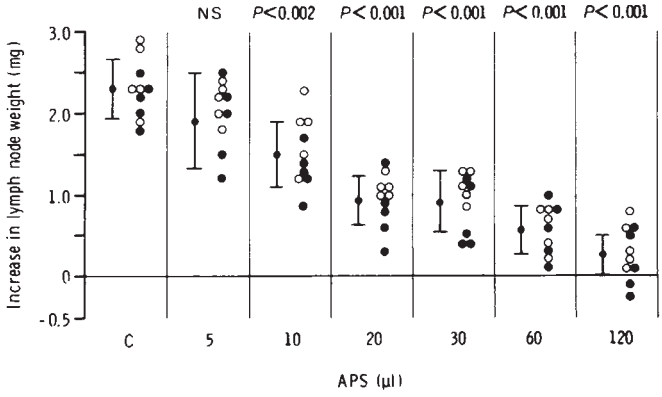

Fig. 3 Dose response of APS in a GvH reaction. (CBA × C57BL/10)F_1 mice received spleen cells from C57BL/10 mice in the left footpad and spleen cells from (CBA × C57BL/10)F_1 animals in the right. Various doses of APS were injected s.c. into the nuchal region on day 0, immediately after the footpad inoculation of 5×10^6 spleen cells. The weights of the popliteal lymph nodes were measured 4 days after inoculation of the cells. Data points show increase in weight of the left popliteal node in individual animals treated with APS or control serum (C) as derived from CBA mice (○) or NZW rabbits (●). Bars represent the mean increase in weight (±s.d.) per group. (For statistical evaluation see Fig. 2 legend.)

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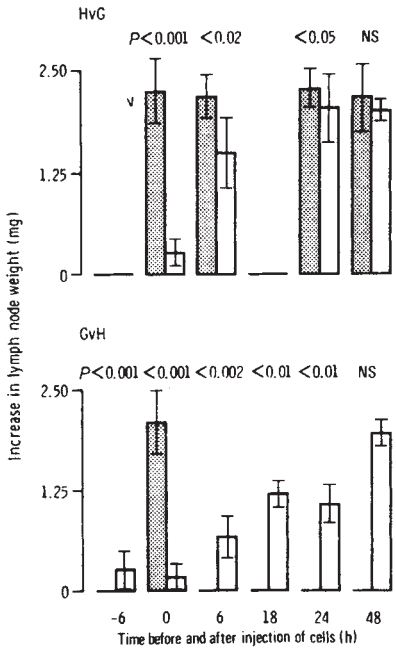

Fig. 4 The effect of a single dose of APS on HvG and GvH reactions in mice, in relation to the time of APS administration. Groups of CBA and (CBA×C57BL/10)F_1 mice (five per group) received footpad inoculations of 5×10^6 donor spleen cells; APS was injected at different time intervals before or after cell inoculation. Lymph node weights were determined on day 3 (HvG) or day 4 (GvH) after cell inoculation. Columns represent the mean increase in weight (±s.d.) per group of the left popliteal lymph nodes in animals treated with APS (open columns) or control serum (shaded columns). (For statistical evaluation see Fig. 2 legend.)

adding PGE_2 to the cultures. Thus, it was suggested that PGEs may be negative (that is, inhibiting) mediators of CMI development.

In contrast to these *in vitro* findings, our *in vivo* data indicate that PGE is required as a positive (augmentative) mediator substance during the induction phase of CMI. These contrasting results could be reconciled if the effect of prostaglandin was governed by a bell-shaped dose–response relationship. According to our hypothesis, low prostaglandin concentrations would be required to initiate or enhance the induction of CMI responses whereas high concentrations would inhibit this process. Anti-PG antibodies may be more effective than indomethacin in reducing the concentrations of active prostaglandin at the site of action and treatment with such antibodies might thus deprive the immunological microenvironment of agents required for the induction of immune responses.

Our results emphasize the potential importance of anti-PG antibodies as tools for elucidating the effects of different prostaglandins and other EFA derivatives on immune mechanisms. It might also be possible to use such antibodies to correct immunological disorders. However, a shortcoming of the APS used here is its polyvalent nature. Greater specificity could be achieved using monoclonal antibodies, and experiments to explore this possibility are in progress.

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Identification of antigenic determinants unique to the surfaces of cells transformed by Epstein–Barr virus

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Epstein–Barr virus (EBV) transforms human B lymphocytes *in vitro* and is associated with two human lymphoproliferative diseases, Burkitt's lymphoma and infectious mononucleosis. In contrast to the lymphoma, infectious mononucleosis is self-limiting and the finding by Svedmyr and Jondal[1] of cytotoxic T lymphocytes specific for EBV-transformed cell lines in mononucleosis patients' blood during the acute phase suggests that this self-limitation results, in part, from the cell-mediated immune response of the host. These workers termed the structure expressed on EBV-transformed cells and which was recognized by specific, cytotoxic T cells, LYDMA (lymphocyte determined membrane antigen). It has also been shown that with appropriate education in culture, cytotoxic T cells from healthy donors specifically kill autochthonous, EBV-transformed cell lines[2]. These target cells generally do not produce virus and bear no virion-associated antigens. We have now isolated monoclonal antibodies which detect antigenic determinants unique to the surfaces of EBV-transformed cells but are not associated with virus production. Quantitative assays for these determinants indicate that they are expressed strongly on cells transformed *in vitro* by EBV but are expressed at intermediate or undetectable levels on cell lines established from patients with Burkitt's lymphoma. These antigenic determinants may be among those recognized by autochthonous, cytotoxic T cells specific for EBV-transformed cell lines, and if they are, their diminished expression on tumour-derived cells may reflect a selective advantage *in vivo* for those tumour cells.

Spleen cells from C57BL/6 or BALB/c mice immunized by repeated intraperitoneal injections of 10^7 cells of one clone of EBV-transformed human cells were used to generate hybridomas following the method of Kohler and Milstein[3]. A two-step screening was performed to select those hybridomas whose antibodies bound to an EBV-transformed cell clone different from that used for immunizations but not to primary T lymphocytes. Eighteen hybridomas which showed this specificity of binding were isolated, cloned at least three times and injected into pristane-primed mice to form ascites tumours.

The monoclonal antibodies from the 18 different ascitic fluids were first characterized by assaying for their binding to a panel

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Table 1 Binding of monoclonal antibodies to human cell lines

Binding	Description of cell line	Association with EBV	Ref.
Strongly positive	Five clones of cells transformed *in vitro* by EBV from three donors	+	10
	Four cell lines transformed *in vivo* and not associated with Burkitt's lymphoma: WI-L2, RPMI 1788, E.H. IV, CCRF-SB	+	11, 12, 13, 12
Intermediate	Four cell lines established from four Burkitt's lymphoma patients: B35M, B46M, AG876, Raji	+	12, 12, 14, 12
Negative	Three cell lines established from three Burkitt's lymphoma patients: EB-3, Daudi, P3HR-1	+	15, 12, 16
	One EBV-negative Burkitt's lymphoma cell line, BJA-B-1	−	16
	One acute lymphocytic B-cell line, BALL-1	−	12
	One multiple myeloma cell line, RPMI 8226	−	12
	One lymphosarcoma B-cell line, U-698-M	−	12
	One acute lymphocytic T-cell line, CCRF-CEM	−	12
	One acute lymphocytic T-cell line, CCRF-HSB-2	−	12
	One erythroleukaemia cell line, K-562	−	12
	One chronic myelocytic non-B, non-T cell line, NALM-1	−	12
	One human fibroblast strain	−	

The binding of the four monoclonal antibodies to human cell lines was measured with a sheep red blood cell (SRBC) rosette assay using ascites fluid prepared from each hybridoma and diluted from 10^{-1} to 10^{-6} in 10-fold increments[17]. Aliquots (50 μl) of each dilution were incubated with 2×10^5 cells at 4 °C in assay buffer: phosphate-buffered saline, 5.0% fetal calf serum and 0.4 mg ml^{-1} pooled human γ globulin. After 0.5 h, cells were washed twice and pelleted with 5×10^7 SRBC coupled to affinity-purified goat anti-mouse immunoglobulin. After 0.5 h, the cell pellets were resuspended in 50 μl of assay buffer and viewed by light microscopy for rosette formation. The results obtained for binding were classified into three groups by the following criteria: strongly positive = 90% of the test cells formed rosettes down to dilutions of 10^{-4}–10^{-5}; intermediate binding = 10–30% test cells formed rosettes down to dilutions of 10^{-3}–10^{-4}; negative binding = no rosettes formed at dilutions $>10^{-1}$.

of human cell lines. Four of these antibodies have the same specificities. The data in Table 1 indicate that only cell lines transformed by EBV bind these four monoclonal antibodies; cell lines transformed *in vitro* or those transformed *in vivo* but not associated with Burkitt's lymphoma bind them strongly; cell lines from patients with Burkitt's lymphoma bind them at intermediate or undetectable levels. Among the cell lines which also fail to bind these antibodies are four derived from B-cell tumours which are not associated with EBV.

Binding of these four antibodies to several of the lines listed in Table 1 was measured quantitatively using a fluorescence-activated cell sorter and the results showed that in identical conditions 85–97% of cells in the strongly positive populations bound the antibodies, compared with 10–30% and 2–5% of the intermediate and negative populations, respectively. In addition, 2–5% of the negative populations bound an anti-H-$2K^k$ monoclonal antibody which serves as a background for nonspecific binding in this assay. Among the fraction of cells scored as binding, the median fluorescent cell in the strongly positive populations bound five times as much label as did the median fluorescent cell in the intermediate populations. Monoclonal antibodies with the above binding specificity will be termed anti-EBVCS.

EBVCS determinants are not associated with virus production and are therefore different from the virus-associated cell-surface antigen, MA[4]. The cell line, P3HR1, in which ~5% of the cells permit viral maturation and express MA, does not express EBVCS determinants, nor does the B95-8 cell line in which 4–8% of the cells permit viral maturation and express MA. These two cell lines were negative for EBVCS determinants using both a rosetting assay and a direct immunofluorescence assay (Table 1 and unpublished data). In addition, clones of cells transformed *in vitro* by EBV in which fewer than 3 cells per 10^5 release virus per 24 h (ref. 5) are uniformly positive for expression of EBVCS (Table 1).

To determine whether EBVCS determinants are present on or can be induced in primary human lymphocytes, we have assayed for their expression on untreated lymphocytes and on lymphocytes exposed to EBV, concanavalin A (Con A) and pokeweed mitogen (PWM). Between 0.5 and 4% of freshly isolated human lymphocytes bind anti-EBVCS antibodies as well as an anti-H-$2K^k$ monoclonal antibody. Primary lymphocytes lose this initial background of binding after several days in culture. The data in Fig. 1 indicate that only lymphocytes exposed to EBV express EBVCS determinants, detectable within 48 h after infection. Neither blast transformation induced by Con A nor blast transformation and immunoglobulin secretion induced by PWM in lymphocytes[6] in the absence of

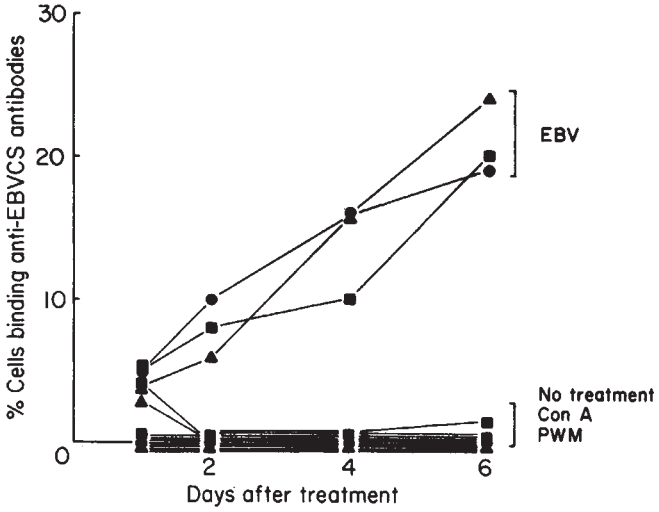

Fig. 1 Lymphocytes isolated from the peripheral blood of healthy donors were infected with the B95-8 strain of EBV at an MOI 1, or were treated with PWM and Con A at concentrations of 10 μg ml^{-1} and 2 μg ml^{-1}, respectively. All cells were cultured in RPMI 1640 medium + 10% fetal calf serum. On 1, 2, 4 and 6 days after treatment, a sample of cells from each culture was assayed for the binding of anti-EBVCS antibodies (IgG1A (▲), IgG1B (■) and IgM (●)) using an indirect immunofluorescence assay. For each assay, 4×10^5 cells were incubated at 4 °C with a 10^{-3} dilution of a hybridoma's ascites fluid in 100 μl of assay buffer (described in Table 1 legend). After 0.5 h, the cells were washed, incubated with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse immunoglobulin at 4 °C for 0.5 h, washed again and dried on glass slides. Cells were fixed with methanol, counterstained and then scored under UV epifluorescence microscopy. Ascites fluid raised from an anti-H-$2K^k$ hybridoma was used to measure nonspecific binding. A cloned line of *in vitro*-transformed cells (THLB1) was included in each assay as a positive control and gave ≥90% fluorescent staining cells. For each point, 100–300 cells were counted and the data were expressed as the per cent of positive staining cells. The immunoglobulin subclass of the four anti-EBVCS antibodies was determined using FITC-goat anti-mouse IgG1, IgG2 and IgM. Although all four EBVCS antibodies were used, results are given for the three which were isolated from separate fusions. The results for the fourth anti-EBVCS, which is an IgM antibody and isolated from the same fusion as the other IgM, are equivalent to those shown. The mitogenic response of primary lymphocytes to Con A and PWM treatment was established by measuring stimulation of DNA synthesis. 10^6 cells from each culture were pulsed with 0.5 μCi ^{3}H-thymidine (50 Ci m mol^{-1}) in 1.0 ml for 24 h beginning on day 3. Cells were lysed and incorporated ^{3}H-thymidine was determined by trichloroacetic acid precipitation. Fourfold more ^{3}H-thymidine was incorporated into the Con A- and EBV-treated cultures than into the untreated culture. The per cent of cells expressing EBNA in the untreated and EBV-infected cultures was assayed as published previously[7] and was found to be 0.0% and 10%, respectively.

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Table 2 EBNA-positive and EBVCS-positive cells form overlapping populations

Antibody used in assay	Unseparated lymphocytes (%)	T cells (%)	B cells (%)
Anti-EBNA	14	1.5	66
Anti-H-2K^k	0	0	3.3
Anti-EBVCS-IgG1A	16	0.5	63
Anti-EBVCS-IgG1B	11	0.5	63
Anti-EBVCS-IgM	12	1	62

After 5 days in culture, a portion of EBV-infected primary lymphocytes was left unseparated and a portion was separated into T- and B-cell populations using Petri dishes coated with rabbit anti-human Fab antibodies[8]. These three populations of cells were scored for EBVCS determinants by indirect immunofluorescence as described in Fig. 1 legend and for EBNA by anti-complement immunofluorescence[7]. 100–300 cells were counted for each assay and the data expressed as the per cent of cells exhibiting immunofluorescence. THLB1 cells were included in each assay and ≥90% of the cells stained positively for both EBNA and EBVCS.

EBV leads to detectable expression of EBVCS determinants.

The observed association between transformation by EBV and expression of EBVCS determinants indicates that those infected cells which develop expression of EBNA[7], the EBV-induced nuclear antigen, may also be those which express EBVCS. To test this prediction, primary lymphocytes were exposed to EBV, cultured for 5 days and a portion of the cells separated into immunoglobulin-positive and -negative populations by binding them to plastic dishes coated with rabbit anti-human Fab antibodies[8]. Assay of unseparated populations and the immunoglobulin-positive and -negative populations (Table 2) indicates that those cells which express EBVCS determinants are separated as B cells along with those which express EBNA and that the two antigen-expressing populations are overlapping and may be identical.

To identify the molecule(s) which carries the EBVCS determinants, cells were labelled with ^{125}I using lactoperoxidase, and immunoprecipitates of extracts of the labelled cells were resolved on polyacrylamide gels. The results depicted in Fig. 2 indicate that one of the two IgG class antibodies precipitates a polypeptide with an apparent molecular weight of 45,000 from only the EBVCS-positive cell line. Although this polypeptide is likely to be the molecule which carries EBVCS determinants, its certain identity awaits further experimentation.

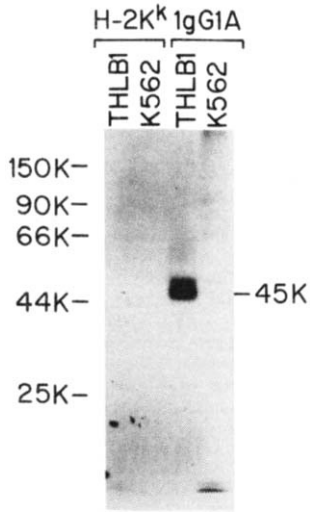

Fig. 2 Immunoprecipitations using monoclonal antibodies were carried out on lysates prepared from 2×10^7 THLB1 (EBVCS positive) and 2×10^7 K562 (EBVCS negative) cells labelled with ^{125}I and lactoperoxidase as described previously[18] with the following modification: 2.5 μl of ascites fluid from either the IgG1A (anti-EBVCS) or anti-H-2K^k and 2.0 μl of affinity-purified goat anti-mouse immunoglobulin (6.0 mg ml^{-1}) were used for each precipitation before addition of formalin-fixed *Staphylococcus aureus*. Immunoprecipitated proteins were electrophoresed in an SDS-polyacrylamide gel and visualized using autoradiography as described elsewhere[18]. The molecular weight markers were identified by staining the gel with Coumassie blue before preparing it for autoradiography. The markers consist of *Escherichia coli* RNA polymerase (150 K, 90 K), bovine serum albumin (66 K), ovalbumin (44 K) and chymotrypsinogen (25 K).

Four monoclonal antibodies bind to the same spectrum of cells and identify EBVCS determinants unique to EBV-transformed cells. The use of these monoclonal antibodies to assay these determinants quantitatively indicates that EBVCS determinants are expressed at reduced levels on cell lines established from Burkitt's lymphoma patients relative to their expression on all other EBV-transformed cell lines tested. The EBVCS determinants are among possible determinants recognized by autochthonous, cytotoxic T cells specific for EBV-transformed cells and may therefore be equivalent to LYDMA. The possibility of this equivalence is supported by the finding that EBVCS and LYDMA are expressed with the same time course in lymphocytes infected with EBV *in vitro* (Fig. 1 and ref. 9). If the EBVCS determinants are used by cytotoxic T cells to identify their targets, the reduced expression of EBVCS on Burkitt's lymphoma cell lines is consistent with these tumour cells having been selected *in vivo* to survive that cytotoxic response.

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Specificity of T-cell clones illustrates altered self hypothesis

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Cytotoxic T lymphocytes (CTLs) recognize foreign antigens in the context of self major histocompatibility antigens. In the mouse this phenomenon is called H–2 restriction as H–2 is the major histocompatibility complex. Two theories have been proposed to explain the dual requirement for CTLs to kill specifically target cells: (1) the two-receptor hypothesis proposes that T cells have an anti-self H–2 receptor and a distinct anti-foreign (anti-X) receptor[1,2]; (2) the altered self hypothesis proposes that T cells recognize complex antigens or interaction antigens created by the physical interaction of self H–2 molecules and X in the target cell membrane[3,4]. Here we report the isolation of CTL clones which recognize H–2^k-plus-X and cross-react with H–2^d-plus-Y. Because the clones recognize two pairs of antigens and not any combination, for example, H–2^k-plus-Y, they support very strongly the principle of altered self recognition, that is, that only the complex of H–2 and foreign antigen is recognized and not either component independently.

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In one set of experiments we have isolated a number of cloned, diploid, H-2^k/H-2^d CTL lines which were originally selected *in vitro* to recognize H-2^k-plus-minor antigen 1 but which also recognize H-2^d-plus-minor antigen 2. Radiation chimaeras ($F_1 \rightarrow$ parent) were constructed by reconstituting lethally irradiated B10.D2 (H-2^d) mice with (B10.BR × B10.D2)F_1 H-2^k/H-2^d bone marrow cells. When immunized *in vivo* and *in vitro* with minor H antigen-different H-2^k/H-2^d (BALB.K × BALB/c)F_1 cells, these chimaera cells show a 10- to >50-fold preference for reacting to H-2^d (that is, BALB/c) target cells. Therefore, as has been shown previously[5-7], these chimaeras have a self or thymic preference in H-2 restriction. The H-2^k-restricted CTLs from one chimaera which had a 20-fold H-2^d-restriction preference were selected by stimulation in mixed lymphocyte culture (MLC) with irradiated BALB.K cells on days 0 and 9 of culture. On day 14 these cultures reacted well with BALB.K targets but significantly also reacted strongly to BALB/c targets. The cultured cells were subsequently stimulated in MLC with irradiated BALB/c stimulator cells on days 19 and 31 and were eventually cloned in limiting dilution (see Fig. 1 legend). Seven CTL clones (and subsequently a number of subclones) all with the same cytotoxic specificity were isolated. (The derivation of these clones will be described in detail elsewhere.) Figure 1 shows that the (B10.BR × B10.D2)F_1 CTL clone, cr-15, lyses BALB/c (H-2^d) and BALB.K (H-2^k) target cells very efficiently, but does not lyse either syngeneic (B10.BR × B10.D2)F_1 target cells nor target cells from either parent. That the CTL activity measured on BALB targets is actually true H-2-restricted anti-BALB minor activity is shown by the following cases of 'F_1-specific lysis'. BALB.B (H-2^b), B10.BR and B10.D2 targets are not lysed, but target cells from (BALB.B × B10.BR)F_1 and (BALB.B × B10.D2)F_1 mice are sensitive to lysis. In these F_1 targets, the BALB.B parent provides a minor H antigen and the B10.BR or B10.D2 parent provides the correct restricting H-2 allele.

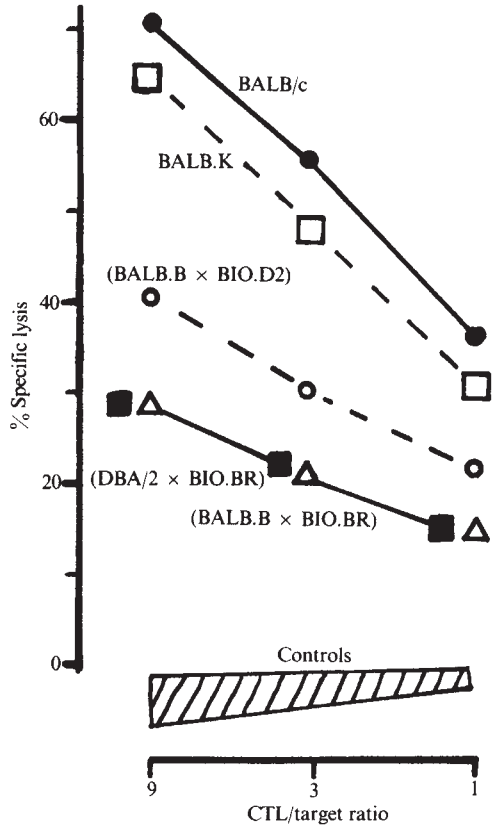

Fig. 1 Cytotoxic activity of CTL clone cr-15 on various labelled target cells. A radiation chimaera, (B10.BR × B10.D2) → B10.D2, was primed *in vivo* 8 weeks following the irradiation by an injection of (BALB.K × BALB/c)F_1 spleen cells. After 8 weeks, spleen and lymph node cells from the chimaera were placed in MLC with irradiated BALB.K spleen cells as stimulators in MLC medium[15]. Cells were stimulated once more with BALB.K stimulators and twice with BALB/c stimulators and were cloned on day 40 of culture according to the technique of Glasebrook and Fitch[16]. Long-term MLC cells were suspended at 3 cells ml^{-1} with irradiated BALB/c spleen cells (3×10^6 cells ml^{-1}) in MLC medium containing 40% conditioned medium from concanavalin A-activated mouse spleen cells plus 50 mM α-methylmannoside. Aliquots (0.1 ml) were cultured in the wells of Coster 3596 plates. Cytotoxic clones were maintained by weekly subculture in conditioned medium plus irradiated stimulator cells. Five months after the initial cloning, cr-15 CTLs were assayed for lysis of ^{51}Cr-labelled spleen cell targets which had been cultured for 3 days with lipopolysaccharide. Targets were: BALB/c (●), BALB.K (□), (BALB.B × B10.D2)F_1 (○), (BALB.B × B10.BR)F_1 (■), (DBA/2 × B10.BR)F_1 (△). Control targets which were not sensitive to lysis included B10.D2, B10.BR, BALB.B, DBA/2 and (DBA/2 × B10.D2)F_1. Six independent cr clones isolated at the same time as cr-15 were also able to kill both BALB/c and BALB.K targets. Furthermore, all four subclones of cr-15 had the same specificity.

The most important question regarding the specificity of these CTL clones is whether the H-2^k-restricted and H-2^d-restricted minor H antigens recognized are the same or different. Clearly both antigens are present in the BALB non-H-2 background, but the B10 and BALB strains differ by >40 minor H alleles[8,9]. We have been able to separate the antigens genetically to show that in fact the H-2^k- and H-2^d-restricted antigens are different: cells from the DBA/2 (H-2^d) strain and (DBA/2 × B10.D2)F_1 mice are not sensitive to lysis by these CTL clones, whereas (DBA/2 × B10.BR)F_1 target cells are lysed . Thus, we conclude that the H-2^d-restricted minor H antigen is present in BALB but not in DBA, whereas the H-2^k-restricted antigen is present in both BALB and DBA mice.

Further tests with these clones (Table 1) suggest that the H-2^k restriction maps to K^k as C3H (K^kD^k) targets are sensitive to lysis whereas C3H.OH(K^dD^k) targets are not. The H-2^d restriction of the cr CTL clones probably maps to D^d as BALB/c (K^dD^d) targets are sensitive to lysis while BALB.B (K^bD^b) and BALB.HTG (K^dD^b) targets are not. We conclude that these CTL clones recognize minor antigen 1-plus-K^k and minor antigen 2-plus-D^d.

The binding specificity of CTLs can also be studied in cold target competition assays in which the release of ^{51}Cr from one target is studied in the presence of an excess of unlabelled competitor cells. For CTL clone cr-15, the rate of lysis of ^{51}Cr-labelled BALB/c or BALB.K targets is inhibited by either BALB/c or BALB.K competitors (Table 2). This result reaffirms the clonality of the CTLs and suggests that the two targets cells interact with the same receptor. Unlabelled DBA/2 and BALB.B targets do not inhibit the CTLs. We consistently found that cold BALB/c cells are more efficient competitors than cold BALB.K even when BALB.K is the labelled indicator cell. With ^{51}Cr-labelled BALB/c as the indicator cell, BALB.K cells are 5–10-fold less efficient inhibitors than BALB/c cells. Thus, the CTLs seem to have a higher affinity for the H-2^d target.

Table 1 Cytotoxic specificity of cr-15 CTLs

LPS blast target	H-2 alleles K	I	D	% Specific lysis at CTL target ratio of: 21:1	2.3:1
(B10.BR × B10.D2)F_1	*k/d*	*k/d*	*k/d*	−2	0
BALB/c	*d*	*d*	*d*	64	51
BALB.K	*k*	*k*	*k*	62	46
BALB.B	*b*	*b*	*b*	−2	0
BALB.HTG	*d*	*d*	*b*	−3	0
C3HeB/FeJ	*k*	*k*	*k*	71	53
C3H.OH	*d*	*d*	*k*	−4	0

Cloned cr-15 CTLs were assayed for lysis of ^{51}Cr-labelled lipopolysaccharide (LPS)-induced spleen cells derived from various strains of mice.

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Table 2 Cold target inhibition studies of the cr-15 cloned CTL line

Unlabelled cells added	% Specific ^{51}Cr release from: BALB/c	BALB.K
None	35	26
BALB.B	35	28
DBA/2	37	26
(B10.BR × B10.D2)F_1	36	27
BALB/c	2	−2
BALB.K	16	0

^{51}Cr-labelled target cells (4×10^4) of BALB/c or BALB.K origin were mixed with 2.7×10^4 cloned cr-15 CTLs in 1 ml of assay medium in the presence or absence of 1.8×10^6 unlabelled cells from the various strains shown. The cytotoxic assay was incubated for 3.5 h. Labelled and unlabelled target cells were spleen cells cultured for 3 days with a mitogenic dose of lipopolysaccharide.

We have isolated other CTL lines having a similar cross-reactive specificity. For example, an *in vitro* line of (C3H × DBA/2)F_1 (H-2^k/H-2^d) CTLs from normal mice binds to and lyses target cells expressing B10 minor antigen-plus-H-2^d (that is, B10.D2 cells) and trinitrophenyl-bovine serum albumin (TNP-BSA)-coupled C3H (H-2^k) cells[10,11] but does not recognize either B10 minor-plus-H-2^k or TNP-BSA-plus-H-2^d (ref. 12).

The fact that CTLs recognize X-plus-K and Y-plus-D but not the other combinations (X-plus-D, Y-plus-K, X-plus-Y or K-plus-D) shows that H-2-restricted T cells do not recognize foreign antigen and self H-2 as separate entities. The cross-reactivity pattern cannot be explained by cross-reactivity at either the anti-self or anti-X sites which have been postulated to exist in two receptor models. The T cells are specific for the complex antigen[13]. Our results agree with those of Kappler *et al.*[14] who fused two H-2-restricted cells but did not detect reshuffling of the anti-self and anti-foreign antigen specificities. It seems highly likely that the principle of the altered self hypothesis is correct, that is, self H-2 antigens modify the antigenicity of foreign antigens[3,13]. In this way, CTLs will recognize antigen only when it is associated with the plasma membrane of target or stimulator cells. The probable function of H-2 restriction is to focus the attention of T cells on to membrane-bound antigens.

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Multiple conductance states of single acetylcholine receptor channels in embryonic muscle cells

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Acetylcholine (ACh) activates in the synaptic membrane of skeletal muscle an inward current composed of many elementary currents[1,2]. High resolution current measurements in adult frog muscle have shown that the elementary current is a pulse-like event of unit amplitude, indicating that ACh opens ion channels which have only two conductance states, fully open or closed[3]. We now present evidence for a third conductance state. In the membrane of uninnervated embryonic rat muscle we observe that ACh activates two independent classes of currents of different amplitude and average duration, apparently arising from two populations of ACh receptor (AChR) channels. The currents from both classes show, at low incidence, transitions between a main level and a sublevel of lower amplitude. From this we conclude that AChR channels in embryonic muscle adopt, in addition to a 'main' conductance state, a 'substate' of lower conductance.

Single channel currents activated by ACh were recorded from cell-free membrane patches isolated from spherical 'myoballs'. Most experiments were performed at low temperatures (5–8 °C) using the 'outside-out' patch configuration[4], in which the extracellular membrane face is exposed to the bath solution (Fig. 1*a*). Following addition of ACh to the bath solution at 1–2 μM the membrane current increases from the resting level in a stepwise fashion by superposition of elementary current contributions (Fig. 1*a*). Figure 1*b* shows the waveforms of individual current contributions recorded from one patch at −100 mV. They occur predominantly as rectangular pulses of current of two different amplitudes (Fig. 1*b*, upper record). The mean amplitudes of these 'main' current levels are 4.3 pA and 2.9 pA, respectively (Fig. 1*c*). A small percentage (≤10%) of currents, however, has a more complex waveform, representative examples of which are shown in the middle and lower records of Fig. 1*b*. The membrane current jumps from either of the two main levels to a smaller 'sublevel' of 1.1 pA average size.

The amplitude and duration of the sublevel were measured from that class of currents where the main level was of the lower amplitude, because in all patches examined this is the more frequently occurring event. In the patch described above where the larger currents made up nearly 40% of all recorded currents, comparison of the sublevel amplitudes of the two classes (Fig. 1*b*, lower trace) showed that they were not significantly different, both having averages of 1.1 pA. This indicates that the size of the sublevel does not depend on the preceding main level. When the complex current waveforms described above were examined at higher time resolution (4 kHz bandwidth) no transition of the current to the baseline was seen between the two levels. At this time resolution the coincidental occurrence of two individual currents within 0.5 ms would have been detected as separate events. Following the initial transition from the main level to the sublevel the current jumps either back to the resting level or switches between the sublevel and the main level (Fig. 1*b*, middle traces). The frequency of occurrence of these latter complex waveforms where the current fluctuates repeatedly between the main and the sublevel, is three orders of magnitude larger than expected by unresolved coincidence of independently occurring events. These observations are consistent with the view that the complex current waveforms represent transitions of AChR channels between a main conductance state and a substate of lower conductance.

Current–voltage relationships measured in one patch in the potential range between −50 and −120 mV (Fig. 2*a*) indicate that the extrapolated reversal potential for the three current levels is close to 0 mV (Fig. 2*b*). The substate, in this case, has a conductance of 10 pS; the conductances of the two main states are 25 pS and 35 pS, respectively.

The cation selectivity of the substate seems to be similar to that of the two main states. When Na^+ in the bath solution was

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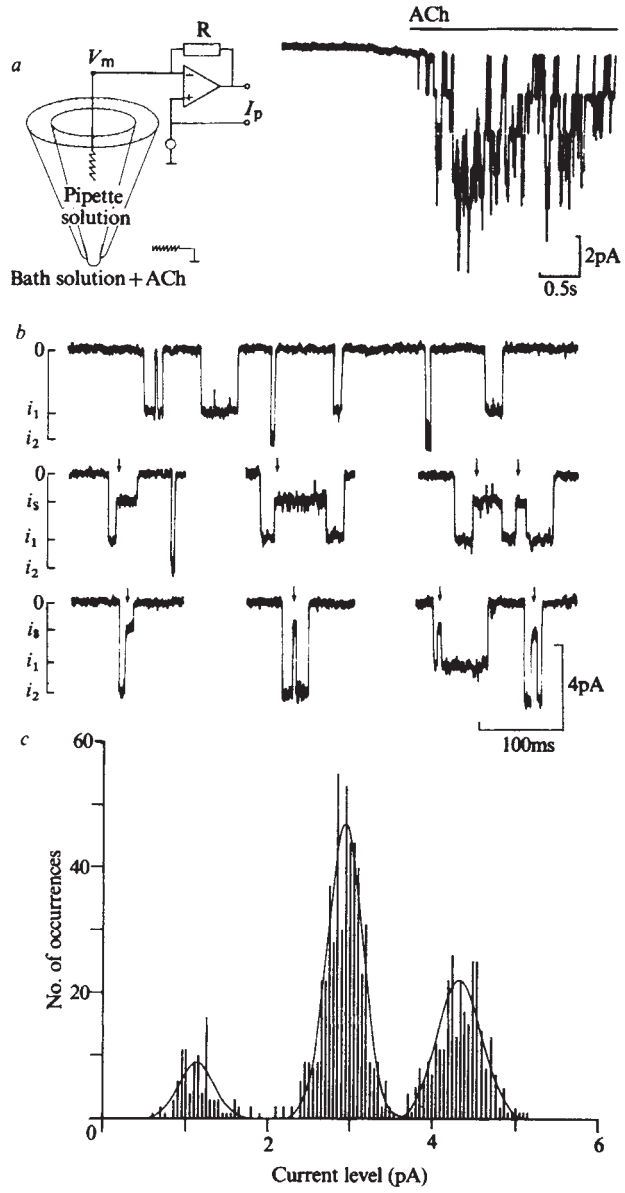

Fig. 1 AChR channel currents in myoball sarcolemma. The methods used in preparing myoballs and techniques of high resolution recording and patch isolation are described in detail elsewhere[4]. *a*, The figure on the left shows schematically the current-recording configuration used to study isolated membrane patches. Outside-out patches were formed according to the following procedure. A high pipette–membrane seal (giga-seal) was obtained on the muscle cell, the initial membrane patch was disrupted by suction and the pipette then slowly withdrawn from the cell. This resulted in the formation of a new membrane patch spanning the pipette tip with its extracellular side facing the bath solution (in mM: 150 NaCl, 1 $MgCl_2$, 1 $CaCl_2$, 3 KCl and 10 HEPES, *p*H 7.2) and its cytoplasmic side facing the pipette solution (in mM: 150 KCl, 3 NaCl, 1 $MgCl_2$, 1 EGTA and 10 HEPES, *p*H 7.2). The membrane potential V_m is defined as the potential of the pipette solution with respect to the bath solution. The patch current I_p is shown as a downward deflection when it flows from the bath solution into the pipette solution. Outside-out membrane patches were preferred for this study because they were typically more stable than cell-attached membrane patches, allowed ACh concentration to be adjusted during the measurements and permitted channel kinetics to be slowed by cooling to low temperatures without altering transmembrane ion gradients. The figure on the right shows the rapid activation of AChR channel currents in an outside-out patch following application (indicated by the bar) of 2 μM ACh to the bath solution, at a potential of −70 mV, 8 °C; 400 Hz filtering. *b*, Oscilloscope traces showing AChR channel currents recorded at low frequency from an isolated patch of sarcolemma. The top trace is a continuous record and indicates the typical current behaviour. Two distinct classes of elementary currents (i_1, i_2) are evident. A small proportion of currents from both classes display more complex waveforms and examples are shown in the middle and lower traces. Some currents jump down to a sublevel (i_s) of ~1 pA and then either return to the resting level or jump back to the original main level. A smaller proportion of events show repetitive fluctuations between the two levels (last example in middle trace). In none of the 809 events recorded in this experiment did the sublevel precede the initial jump to the main level. In two cases the sublevel appeared in apparent isolation. Membrane potential −100 mV, temperature 8 °C. Day 14 myoball, 4 days after colchicine treatment; 1,000 Hz filtering. *c*, Histogram showing the relative occurrence of the three different current levels for the patch described in *b*. The curves are gaussian fits to the amplitudes which had mean values of 1.1, 2.9 and 4.3 pA. A total of 809 discrete current steps were recorded; 509 were in the class that had an average amplitude of 2.9 pA and 298 events were of the 4.3 pA class. Of these, 73 and 17 events, respectively, displayed the sublevel of average amplitude 1.1 pA. Three events with an average amplitude of 2.9 pA displayed a sublevel of ~2 pA, but because of the low frequency of occurrence of this level it has not been studied.

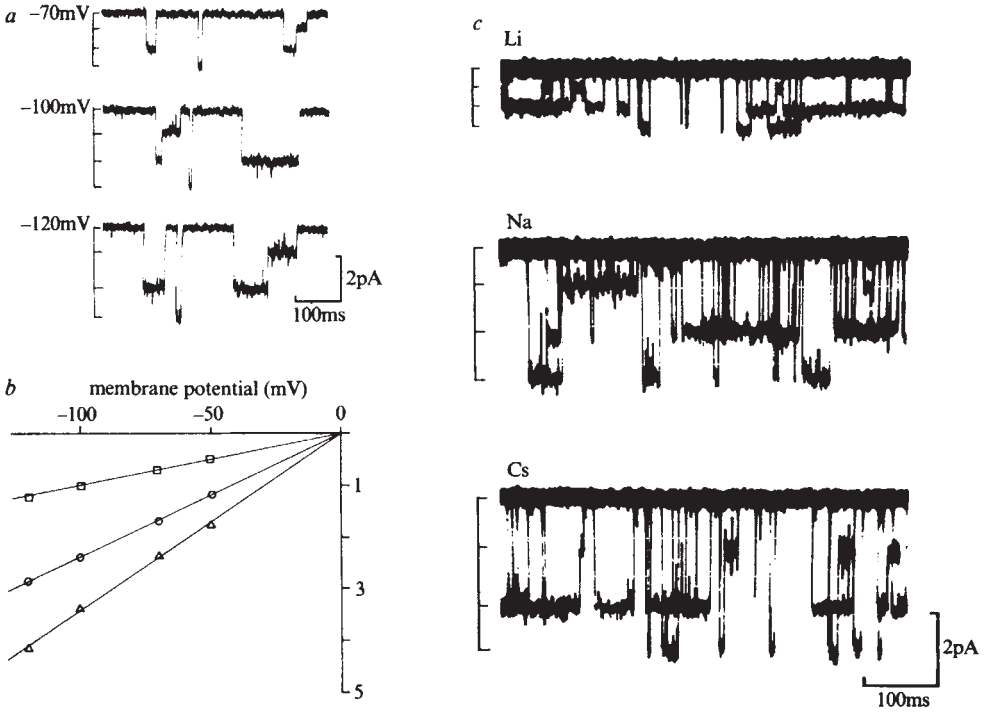

Fig. 2 Voltage dependence and ion selectivity of the three main conductance states of AChR channels. *a*, Current traces recorded from the same patch of myoball sarcolemma at different membrane potentials. ACh concentration 1 μM, temperature 6.5 °C. Day 13 myoball, 3 days after colchicine treatment; 1,000 Hz filtering. *b*, Current–voltage relationship for the three current levels measured from the patch described in *a*. Each data point represents the average amplitude of the level determined from 200–500 events. The lines fitted to the data points extrapolated to give a reversal potential of 0 mV and yielded conductances of 10, 25 and 35 pS. *c*, Superimposed current traces recorded from another patch in which recordings were made in a bath solution that contained initially sodium as the predominant cation (150 mM), then caesium and finally lithium. Membrane potential was −100 mV. Temperature 5 °C. Day 11 myoball, 1 day after colchicine treatment; 1,000 Hz filtering. Current–voltage relationships measured in each solution over the voltage range −50 to −120 mV gave conductance values for the three levels of 4, 9 and 14 pS in Li, 8, 19 and 31 pS in Na and 11, 25 and 34 pS in Cs.

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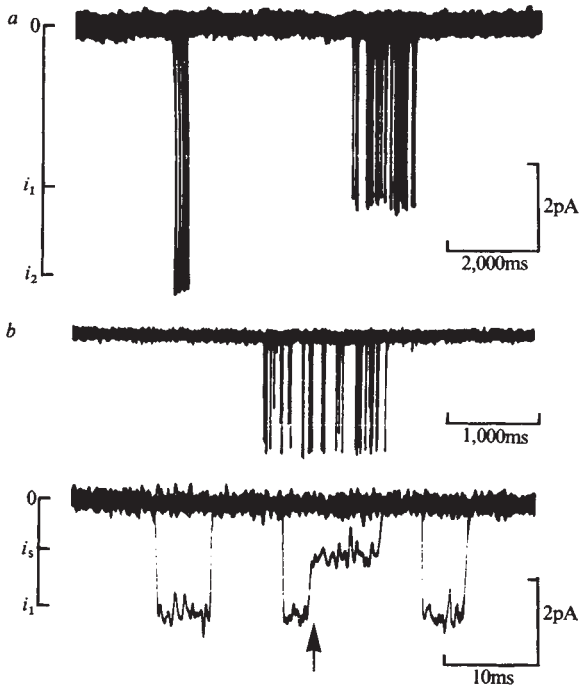

Fig. 3 AChR channel currents activated by desensitizing concentrations of ACh. *a*, Current recording from a sarcolemma patch activated by 10 μM ACh. Two distinct non-interacting bursts of currents were evident that corresponded in their amplitudes to the two main classes evident at low ACh concentrations. Membrane potential was −130 mV, temperature 18 °C. Day 12 myoball, 2 days after colchicine treatment; 400 Hz filtering. *b*, Current recording from another patch. In the top trace a burst of currents corresponding in amplitude to the smaller main level is shown at low time resolution. The bottom trace gives two superimposed traces of the same burst shown at higher time resolution. One distinct transition between the main level and a sublevel was evident and is indicated by the arrow. Membrane potential −100 mV, temperature 18 °C. Day 14 myoball, 4 days after colchicine treatment; 400 and 2,000 Hz filtering.

replaced by Li^+ or Cs^+, the conductance of all three states (Fig. 2*c*) decreased or increased proportionately. The various conductance states of AChR channels however differ in their average lifetime: for the patch described in Fig. 1 the average duration of the larger and smaller main levels was 12 and 36 ms, respectively, whereas the sublevel measured from the same patch had an average duration of 24 ms. However, these durations represent only apparent lifetimes because the fast fluctuations of the single channel current in the time range <1 ms (see accompanying paper[5]) were not taken into account. The ratio of the time the patch current is at the sublevel to the time it is at either the main or the sublevel varied between 0.02 and 0.12 in six different patches, indicating that the probability of the open channel adopting the substate is low. It is further reduced when the temperature is increased: at 18 °C current sublevels are only measurable as short notches (<5 ms) in the channel's closing time course. On the other hand, increasing the membrane potential from −70 mV to −120 mV (temperature 5 °C) increased the probability of the open channel adopting the substate from 0.05 to 0.15 (one patch). When channels have adopted the substate, the probability that they either close completely or open fully was similar. For example, in the patch illustrated in Fig. 1 the ratio of transitions back to the main state to transitions to the closed state was 1.3. Apparent transitions between the two main current levels are also observed, but their average number is not significantly different from that expected by unresolved random coincidence of two main unit current events.

These findings suggest that ACh activates two independent classes of channels in embryonic rat muscle that are distinguished by a slightly different main conductance. Both can adopt, with low probability, a similar substate of lower conductance.

This view is further supported by experiments where channels were activated by ACh at concentrations >2 μM where single channel currents appear in bursts. Bursts reflect sequential open–close transitions of the same AChR channel[6]. In embryonic muscle they fall into two main classes with respect to the size of their current pulses, corresponding to the two classes observed at low ACh concentration (Fig. 3*a*). Transitions between these two main current levels were never observed during a burst. However, transitions between the main level and the sublevel do occur, as shown in Fig. 3*b*. The upper trace shows a burst of current pulses at low time resolution, consisting of 25 well separated current events. At high time resolution two events have a complex shape characterized by a transition from the main level to the sublevel. One of these events is illustrated in the lower trace of Fig. 3*b*. The average probability of this channel being in the substate (given the channel is in the open state), as determined from eight consecutive bursts, was 0.08. This experiment shows directly that a single AChR channel complex, in the presence of constant ACh concentrations, fluctuates between at least three states: a main open state, a substate which is adopted at low probability, and the closed state(s).

The AChR complex purified from *Torpedo* electroplaque is a pentameric protein complex comprised of five homologous subunits[7] which span the membrane[8]. In lipid bilayers it can form a unit conductance channel which has a similar conductance to that of the AChR channel in rat muscle[9]. The observation that ACh-activated channels in embryonic muscle cells can adopt several conductance states could indicate that these subunits rearrange themselves to form the different open states of a channel. Similarly the two independent classes of channels may represent two different aggregation states of the same set of subunits.

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Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels

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Acetylcholine-like drugs cause ion channels in the skeletal muscle endplate to open briefly[1], producing, at random intervals, rectangular pulses of current with constant amplitude but random duration, that can be recorded by the patch clamp method[2,3]. However, even when the agonist concentration is so low that channel activations are very well separated, we have observed, with high resolution methods[4], that openings may be interrupted by shut periods (gaps) so brief that they are very unlikely to arise from two independent channel activations. This sort of behaviour has been predicted on the basis that two or

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more openings might occur during the time for which the receptor remains occupied by agonist[5,6]. If this were correct, important new information about agonist activation of ion channels could be obtained from measurements of the gaps between openings. However, short gaps could arise in other ways: for example from brief blockage of the ion channel[7], perhaps by the agonist itself. We now present results obtained with the acetylcholine-like agonist, suberyldicholine (SubCh, 20–100 nM), which suggest that the brief gaps do not result from ion channel block by the agonist itself, but which are consistent with a mechanism in which the channel opens and closes several times during a single agonist receptor occupancy. We have also observed that the number of short (<1 ms) current pulses is greater than we expected.

Single ion channel currents from the perisynaptic region of normal frog (*Rana temporaria*) cutaneus pectoris muscle were recorded at 10–13 °C (ref. 4). The Ringer solution contained (mM) NaCl 115, KCl 2.5, $CaCl_2$ 1.8 and HEPES buffer 3, *p*H 7.2

Figure 1*a* shows the typically low frequency of single channel currents (always $<3\ s^{-1}$). Individual currents are shown, with high time resolution, in Fig. 1*b,c*; they are clearly interrupted by brief gaps. During most of these gaps the current does not reach the resting value; however, the frequency response of the system is such that the channel would have to be shut for nearly 300 μs for this to be attained. We assume that the gaps represent brief, but complete, closures of the ion channel, and Fig. 1*d–g* show how the duration of such closures was estimated. In good records, events with a duration of 50–70 μs could be clearly resolved.

The entire record was digitized so that the duration of all resolvable gaps, as well as openings, could be measured. After this measurement, a safe value was chosen for the minimum resolvable duration, and the record was revised by concatenation of adjacent gaps and openings separated by intervals less than this chosen resolution. Thus an idealized record, with consistent resolution throughout, was obtained for the construction of histograms. All distributions of time intervals were fitted with the sum of one or more exponential functions by the method of maximum likelihood (that is, the actual measured durations were used, not the histogram frequencies).

The distribution of all gaps is exemplified in Fig. 2. In Fig. 2*a*, the time scale extends up to 2 s, and the gap durations are fitted well by an exponential component with a mean of 352 ms. This is presumably the mean interval between independent activations of ion channels. However, the first bin (up to 50 ms) extends a long way off the graph; the distribution of short gaps is shown in more detail in Fig. 2*b*, in which the time scale extends up to only 500 μs. There is a clear exponential component of the gap distribution with a mean, in this example, of 45 μs, which corresponds to the short gaps illustrated in Fig. 1. Values of 45–70 μs were consistently observed in nine other experiments. This fast component represented, in this case, 77 per cent of the total area of the gap duration distribution, corresponding to a total number of 2,231 short gaps (although many of these would

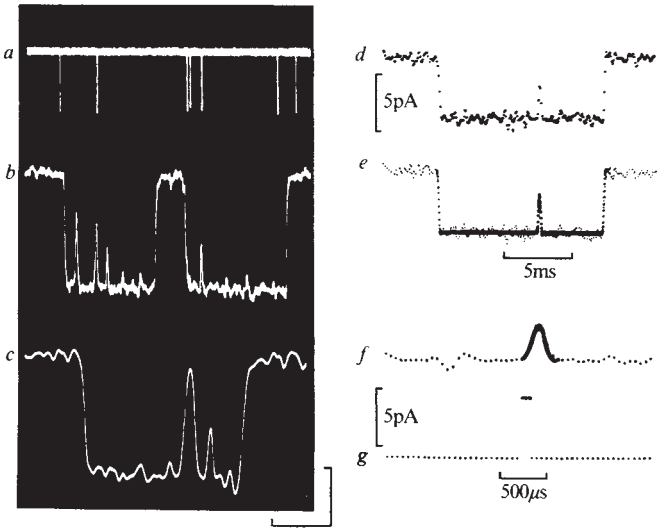

Fig. 1 Examples of single channel currents (*a, b, c*) and the method used to fit their time course (*d, e, f*). Low pass filter set at 4 kHz. *a*, Illustration of low frequency of events (seven in 5 s in this case) with 100 nM SubCh at −123 mV. Calibration bars: 4 pA and 1 s. *b*, Several brief closures interrupting an open channel current; taken from the experiment illustrated in *a*. Calibration bars: 2 pA and 10 ms. *c*, Another group of three openings, at higher time resolution than in *b*; 20 nM SubCh at −128 mV. Calibration bars: 2 pA and 2 ms. *d*, A burst with one resolvable gap, digitized at 16 kHz; 50 nM SubCh at −171 mV. *e*, The same data as in *d* with a fitted line (heavy line) superimposed on it. The response of the recording system to a step input was measured experimentally, and four such response functions, with alternating direction, were convolved to generate the fitted line. *f*, The gap shown in *d* and *e*, with greatly expanded time scale, but the same amplitude scale. A fitted line is superimposed on the data to illustrate the fitting of brief gaps. This fitted line was generated, as explained above, as the expected response of the system to the input shown in *g*. This fit estimates that the burst shown in *d* consists of two openings of length 7.69 and 4.58 ms, separated by a gap of 104 μs, so the total length of the burst is 12.37 ms.

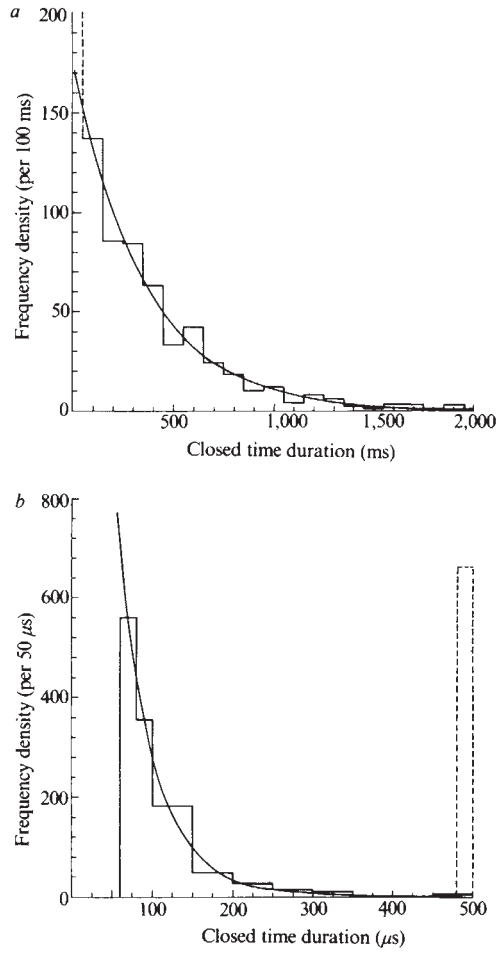

Fig. 2 Distribution of the durations of all closed times (gaps) between open channel currents. Experiment with 20 nM SubCh at −131 mV. Ordinate is frequency density (number of observations per time interval of the specified length). All 1,308 gaps that were measured were simultaneously fitted (as described in the text) with the sum of three exponentials, and the fitted curve was superimposed on the histogram of the observations which is shown with two different time scales in *a* and *b*. In this case we found components with time constants of 352 ms (representing 20.5% of total area under the distribution), 45 μs (76.7% of area) and 0.66 ms (2.8% of area). *a*, Distribution of gaps, shown up to 2,000 ms, with fitted curve. The leftmost bin (dashed line) contains many short gaps, and extends well off the graph. *b*, The same distribution, shown up to 500 μs, only, with fitted curve. The resolution was fixed (see text) at 60 μs in this experiment. The rightmost bin (dashed line) represents all gaps longer than 500 μs. This includes all those shown in *a*.

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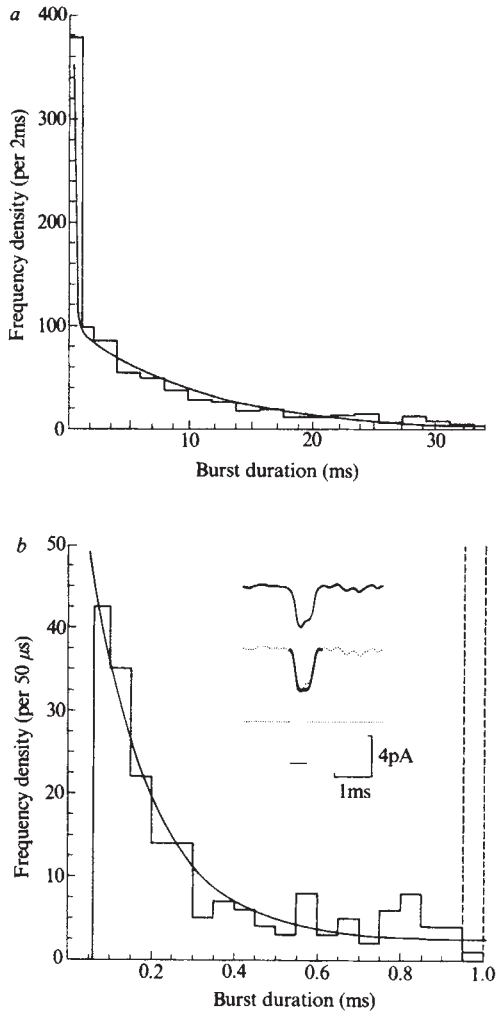

Fig. 3 Distribution of the burst duration, as defined in the text, for the same experiment as shown in Fig. 2. With a resolution of 60 μs and critical gap length of 1.5 ms, there were 683 bursts altogether. The durations of these bursts were fitted (see text) with the sum of two exponentials. In this case we found components with time constants of 10.2 ms, which is similar to the value expected from a noise analysis in the conditions of this experiment, and 0.15 ms. In 12 experiments the average faster time constant was 0.35 ± 0.12 ms. *a*, The distribution of burst length, and fitted curve, shown up to 35 ms. *b*, The same distribution and fitted curve, but bursts up to 1 ms duration only are shown. The rightmost bin (dashed line) represents all bursts longer than 1 ms. The inset shows (top) an example of a brief opening. This is fitted (see Fig. 1, right column) with a superimposed line (middle) calculated as the response to the step input shown (bottom). The amplitude (3.95 pA) was taken as the average of amplitudes of all full size openings previously fitted in this experiment. The duration is 495 μs.

be too short to resolve). In addition we have consistently found a much smaller component (about 2% of total area) with an intermediate time constant of the order of 1 ms, but this will not be considered further here.

The evidence presented above suggests that the 'openings' will contain many unresolved gaps, and are thus not well defined. We therefore define a burst of openings as any series of 'openings' interrupted by gaps that are all less than some critical length (usually 0.5–3 ms). Insofar as this length is very much smaller than the mean time between independent events (352 ms in Fig. 2), the burst duration is a well defined quantity. This occurrence of openings in quick succession was called the Nachschlag phenomenon when it was first observed, and this term seems appropriate, whatever the mechanism responsible for the phenomenon. Figure 3 shows the distribution of burst durations; in Fig. 3*a* the time scale extends to 35 ms, and there are obviously too many short bursts to be fitted by a single exponential distribution. The double exponential distribution shown has a slow component with mean of 10.2 ms, and a fast component with mean of 0.15 ms. The total area under the distribution represents 717 bursts and the slower component corresponds to 73% of the total area in this example, with 20 nM SubCh. Thus we infer, for the experiment illustrated in Figs 2 and 3, that there are, on average 2,231/717 = 3.1 gaps per burst. Similar values (2–4) were found in 10 experiments.

The origin of the short openings illustrated in Fig. 3 is obscure. They may represent a separate type of channel altogether, or perhaps brief openings by channels with only one agonist molecule bound. We shall explore these hypotheses elsewhere.

The most important question that arises is whether the brief gaps result from ion channel block by the agonist, SubCh, itself. Decamethonium (in much higher concentrations) is known to do this[8]. If this were the mechanism, the number of gaps (blockages in this case) per burst should be directly proportional to the agonist concentration. However, the number of gaps per burst with 100 nM SubCh, relative to that with 20 nM, was 0.97 ± 0.11 (three experiments at each concentration), and showed little sensitivity to membrane potential. Therefore, ion channel block by SubCh, in the low concentrations used, cannot explain the brief gaps that we observe. Although it is conceivable that brief closures of the channel might arise from block by some endogenous muscle constituent, or from a mechanism connected with ion permeation, the most plausible alternative to ion channel block is that the closures arise from multiple openings during a single receptor occupancy[5,6]. Suppose, for example, that two agonist molecules must bind sequentially before the channel can open (see, for example, ref. 9). Then (see ref. 6), at low agonist concentration, the mean length of a gap should be approximately $(\beta + 2k_{-2})^{-1}$, and the number of gaps per burst should be approximately $\beta/2k_{-2}$, where β is the rate constant for opening of a doubly occupied receptor–channel complex, and k_{-2} is the microscopic rate constant for dissociation of an agonist molecule. Our results, if interpreted in this way, thus suggest preliminary estimates for β of the order of 10,000–15,000 s^{-1}, with k_{-2} roughly 2,000 s^{-1}. This interpretation of our results with SubCh is not compatible with the common assumption that the agonist binding step is very fast, and that the open–shut conformation change is rate-limiting[10]. We are now investigating whether or not the same can be said of agonists other than SubCh. The noise spectrum expected if this interpretation were correct would consist predominantly of a single component, with a time constant close to the mean length of the burst (slow component), that is, 10 ms in the experiment shown in Fig. 3. But this time constant would not correspond, as is often assumed, to the true lifetime of the open state. In the above example the average burst consists of roughly four openings in quick succession, so the mean open lifetime, $1/\alpha$ (where α is the channel closing rate constant) would be only about 2.5 ms.

Results similar, in some respects, to ours have recently been found (S. G. Cull-Candy and I. Parker, in preparation) for glutamate-operated channels in locust muscle.

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A specific substance P antagonist blocks smooth muscle contractions induced by non-cholinergic, non-adrenergic nerve stimulation

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Nerve fibres containing substance P (SP) are widely distributed in the body[1–3] and seem to innervate autonomic ganglia, blood vessels, epithelial structures and smooth muscle. SP stimulates secretion from exocrine glands, causes vasodilation and contracts non-vascular smooth muscle[4]. The presence of SP in primary sensory neurones has lent support to the view that it is associated with sensory nerve conduction, conceivably as a transmitter[5], and that it is a causative factor in the 'irritative' response to antidromic stimulation of sensory nerves[6]. Gut smooth muscle contracts in response to non-cholinergic, non-adrenergic nervous stimulation[7,8], and it has been suggested that SP acts as an excitatory transmitter in intramural neurones in the gut wall[2,3,9,10]. Recently, a series of synthetic analogues of SP with antagonist activity to SP has been developed[11,12]. We report here that a new analogue, (D-Pro^2, D-Trp^{7,9})-SP, exercises a specific, possibly competitive antagonism to SP. While being a partial agonist it antagonized the contractile response to applied SP and to non-cholinergic, non-adrenergic nerve stimulation in the isolated guinea pig taenia coli and rabbit iris sphincter pupillae muscle, suggesting that SP, or a closely related peptide, is indeed a motor excitatory transmitter. In contrast, (D-Pro^2, D-Trp^{7,9})-SP did not inhibit the contractile response to non-cholinergic, non-adrenergic nerve stimulation of smooth muscle from the guinea pig urinary bladder.

Guinea pig taenia coli preparations, consisting of longitudinal smooth muscle with the attached myenteric plexus[13], were placed in Krebs solution, stored at 4 °C for ~1 h and then mounted vertically on a Perspex holder in a 7-ml organ bath thermostated at 37 °C. One end was attached to a rigid support and the other to a lever connected via a spring to a Grass FT03 force displacement transducer or to a photoelectric transducer for isotonic registration of mechanical activity. The load on the muscle was set at 0.2 g. Platinum ring electrodes were placed around the muscle with a constant electrode distance of 5 mm and the electrodes were connected to a Grass S 4C stimulator for field stimulation with square wave pulses (15 V over the electrodes, 0.5–1 ms duration). The muscles were stimulated with trains of pulses lasting 3 s and with a frequency of 1–5 Hz, the resting period between stimulations being at least 2 min. The mechanical activity of the preparation was continuously recorded on a Grass model 7 or model 5 polygraph. The bathing fluid was a modified Krebs solution of the following composition (in mM): NaCl 133, $NaHCO_3$ 16.3, KCl 4.7, $MgCl_2$ 1.0 NaH_2PO_4 1.4, $CaCl_2$ 2.5 and glucose 7.8. The solution was bubbled with a gas mixture of 7% CO_2 in O_2 giving a *p*H of 7.2–7.3.

The sphincter pupillae muscle of the rabbit iris was excised, opened and mounted as described for the taenia. Mucosa-free strips of the detrusor of the guinea pig bladder were prepared as described in detail elsewhere[14]. The same procedure as above was used to study the motor activity of the sphincter pupillae and the urinary bladder except that they were placed directly in the bath, experiments with sphincter pupillae muscle were run at 35 °C, and the stimulation parameters were 10–20 Hz for 10 s for the sphincter pupillae muscle[15] and a single pulse every 20 s for the urinary bladder. (D-Pro^2, D-Trp^{7,9})-SP was synthesized by techniques analogous to those described elsewhere[11].

The addition of SP and of (D-Pro^2, D-Trp^{7,9})-SP to guinea pig taenia coli produced a contraction which lasted 120–150 s, the tension subsequently returning to baseline (Fig. 1). As illustrated in Fig. 2*a* the contractile effect of the SP analogue required high concentrations. The contractile response to exogenous SP was strongly inhibited by pretreatment with (D-Pro^2, D-Trp^{7,9})-SP (Fig. 1), the blockade lasting for at least 30 min without washing. Washing out the SP analogue resulted in a return of the contractile response to exogenous SP and to electrical stimulation (Fig. 1). The analogue also reduced the contractile response to exogenously applied physalaemin and eledoisin but not that to carbachol, histamine, serotonin, bradykinin, Lys^8-vasopressin or prostaglandin $F_{2\alpha}$ (Fig. 3). In the presence of (D-Pro^2, D-Trp^{7,9})-SP, the dose response curve

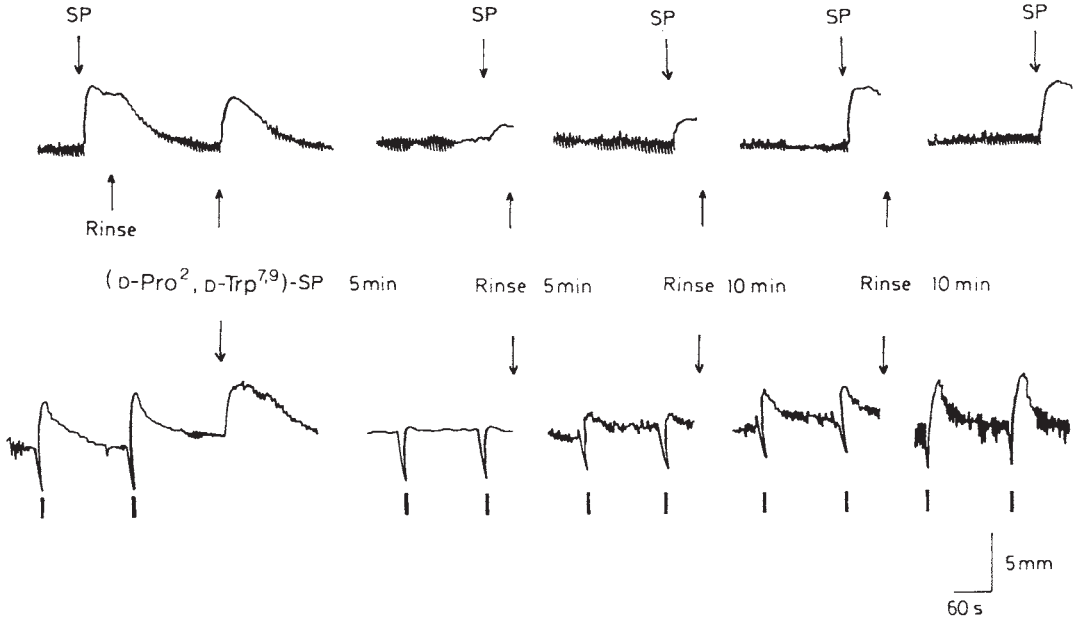

Fig. 1 Effect of (D-Pro^2, D-Trp^{7,9})-SP (10^{-5} M) on the contractile response to exogenous substance P (SP; Peninsula) (5×10^{-9} M) (upper panel) and to the electrically evoked contraction (lower panel) of isolated guinea pig taenia coli. The muscles were electrically stimulated in the presence of atropine (10^{-6} M; ACO) and guanethidine (5×10^{-6} M; Sigma) for 3 s with 3 Hz as indicated by black rectangles. Note the agonist effect of (D-Pro^2, D-Trp^{7,9})-SP, the ensuing blockade of the contractile response to exogenous SP and the reduction of the electrically induced contraction on addition of the SP analogue. Electrical stimulation was applied 5 min after the contractile effect of (D-Pro^2, D-Trp^{7,9})-SP had subsided. The inhibitory effect of (D-Pro^2, D-Trp^{7,9})-SP on smooth muscle contractions can be washed out as illustrated by the recovery of the contractile response of the taenia coli to exogenous SP and to electrical nerve stimulation. Rinsings and time intervals between registrations are indicated. The registrations shown are typical examples of four to six experiments.

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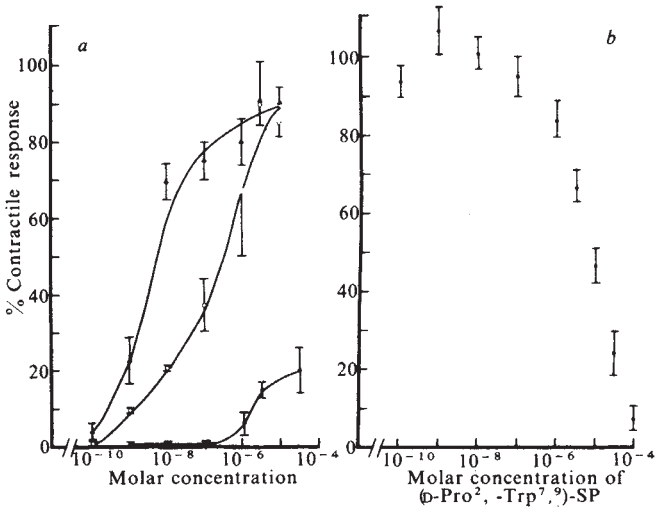

Fig. 2 *a*, Dose–response curves showing the effects of SP alone (▲), of SP in the presence of (D-Pro^2, D-Trp^{7,9})-SP (○), and of (D-Pro^2, D-Trp^{7,9})-SP alone (●) on the tension of the guinea pig taenia coli, expressed as percentage of the contraction induced by carbachol (10^{-5}; Merck-Darmstadt). In the presence of (D-Pro^2, D-Trp^{7,9})-SP (10^{-5} M) the dose–response curve for SP was shifted 2.54 log units to the right, suggesting competitive inhibition. The dose–response curves were constructed by adding step wise increasing amounts of peptides to the bath. Below 10^{-7} M three concentrations of SP were tested on each muscle; above 10^{-7} M only one concentration was tested per muscle. The corresponding concentration for the SP analogue was 10^{-6} M. SP was added in a volume of 200 μl, the SP analogue in 40 μl. Between each addition the bath was rinsed until the tension had returned to base line (usually three rinses, in all 5–10 min). Each value is the mean of 3–10 experiments. Vertical bars give s.e.m. *b*, Effect of increasing concentrations of (D-Pro^2, D-Trp^{7,9})-SP on the contractile response of the isolated guinea pig taenia coli to electrical stimulation (3–5 Hz, 3 s). Atropine (10^{-6} M) and guanethidine (5×10^{-6} M) were present in the bath. New muscles were set up for each concentration tested. Electrical stimulation was applied 5 min after the contractile response to the SP analogue had subsided. The results are expressed as per cent of the contractile responses before adding the SP analogue. Each value is the mean of 4–18 experiments. Vertical bars give s.e.m.

for SP was shifted to the right in a manner suggesting competitive inhibition (Fig. 2*a*). Desensitization with SP is a well known phenomenon[9], but it can be ruled out as an explanation for the inhibitory effect of the SP analogue because high concentrations of SP ($>10^{-6}$ M) could overcome the inhibition, very high concentrations ($>10^{-8}$ M) giving a maximal contractile response.

Electrical stimulation (1–5 Hz, 3 s) of the taenia elicited a strong contraction, and this response was not affected by addition of (D-Pro^2, D-Trp^{7,9})-SP. Following addition of the muscarinic blocker atropine (10^{-6} M) and the anti-adrenergic drug guanethidine (5×10^{-6} M), the taenia responded to low-frequency stimulation (1 Hz, 3 s) by relaxing. With higher-frequency stimulation (3–5 Hz, 3 s), the initial relaxation was followed by a contraction on cessation of stimulation[10]. Both these responses were inhibited by tetrodotoxin, indicating a neuronal mediation[16]. Although relaxation was unaffected, the amplitude of the contraction was greatly reduced by pretreatment with (D-Pro^2, D-Trp^{7,9})-SP (Fig. 1). Interestingly, the dose–response curve revealed that a 10^{-4} M concentration of (D-Pro^2, D-Trp^{7,9})-SP reduced the contraction by more than 90% (Fig. 2*b*).

The iris sphincter pupillae muscle responded to both SP and electrical stimulation with a slow contraction, in the latter case reaching a maximum 30–40 s after cessation of stimulation. The electrically induced contraction lasted for ~150 s. Cholinergic and adrenergic blockade had very little effect on the response to electrical stimulation, but (D-Pro^2, D-Trp^{7,9})-SP caused a dramatic reduction in the contractile response (Fig. 4*a*). The remaining contraction was further reduced by adding tetrodotoxin. As in the taenia coli, the response of the iris sphincter muscle to exogenous SP, physalaemin and eledoisin (but not that to carbachol) was greatly inhibited by pretreatment with (D-Pro^2, D-Trp^{7,9})-SP.

The guinea pig urinary bladder responded to both SP and electrical stimulation with a contraction. Cholinergic and adrenergic blockade had little or no effect on the response to electrical stimulation[14]. Also, (D-Pro^2, D-Trp^{7,9})-SP failed to inhibit the response, which could be completely abolished by tetrodotoxin (Fig. 4*b*).

The results of these experiments indicate that (D-Pro^2, D-Trp^{7,9})-SP is a partial agonist, exercising a specific, possibly competitive antagonism to SP. Interestingly, (D-Pro^2, D-Trp^{7,9})-SP also blocked the effects of physalaemin and eledoisin. Together these peptides have been grouped under the name tachykinins[17,18]; they have the carboxy-terminal three amino acids in common and display smooth muscle stimulating effects[17,18]. Following cholinergic and adrenergic blockade, electrical nerve stimulation still induced a contraction of the guinea pig taenia coli, urinary bladder and rabbit sphincter pupillae muscle. In the taenia coli and sphincter pupillae muscle these contractions were greatly reduced by (D-Pro^2, D-Trp^{7,9})-SP, suggesting that SP is involved as a motor excitatory transmitter. However, the SP analogue did not inhibit the contractile response to electrical nerve stimulation of the urinary bladder following cholinergic and adrenergic blockade. Hence, SP is not in all tissues the only non-cholinergic, non-adrenergic motor excitatory neurotransmitter. SP nerve fibres in the smooth muscle of the taenia coli probably originate from cell bodies in the mysenteric plexus[10,19–21], but their precise nature and mode of action remain to be established. Our results suggest that they are engaged in an efferent motor excitatory capacity although it cannot be excluded that SP is released as a result of antidromic nerve stimulation. The SP fibres in the sphincter pupillae muscle

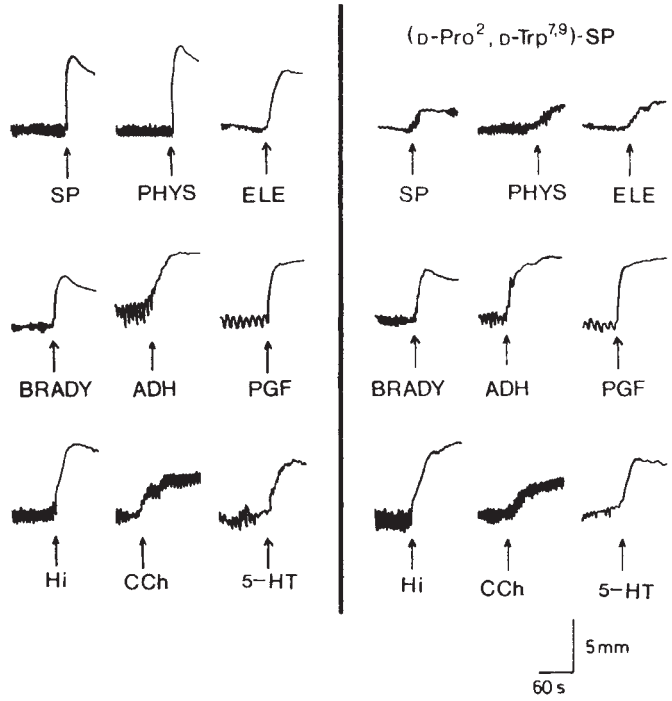

Fig. 3 Contractile response of the isolated guinea pig taenia coli to physalaemin (PHYS), eledoisin (ELE; both Peninsula) at a concentration of 10^{-9} M, SP bradykinin (BRADY; Beckman), histamine (Hi; Merck-Darmstadt), carbachol (CCh) and serotonin (5-HT; Sigma) at a concentration of 10^{-8} M, Lys^8-vasopressin (antidiuretic hormone, ADH; Ferring) and prostaglandin $F_{2\alpha}$ (PGF; Sigma) at a concentration of 10^{-7} M, with (right) or without (left) 10^{-5} M (D-Pro^2, D-Trp^{7,9})-SP in the bath. The contractile effect of the SP analogue was allowed to subside before the other compounds were added, usually 5 min later.

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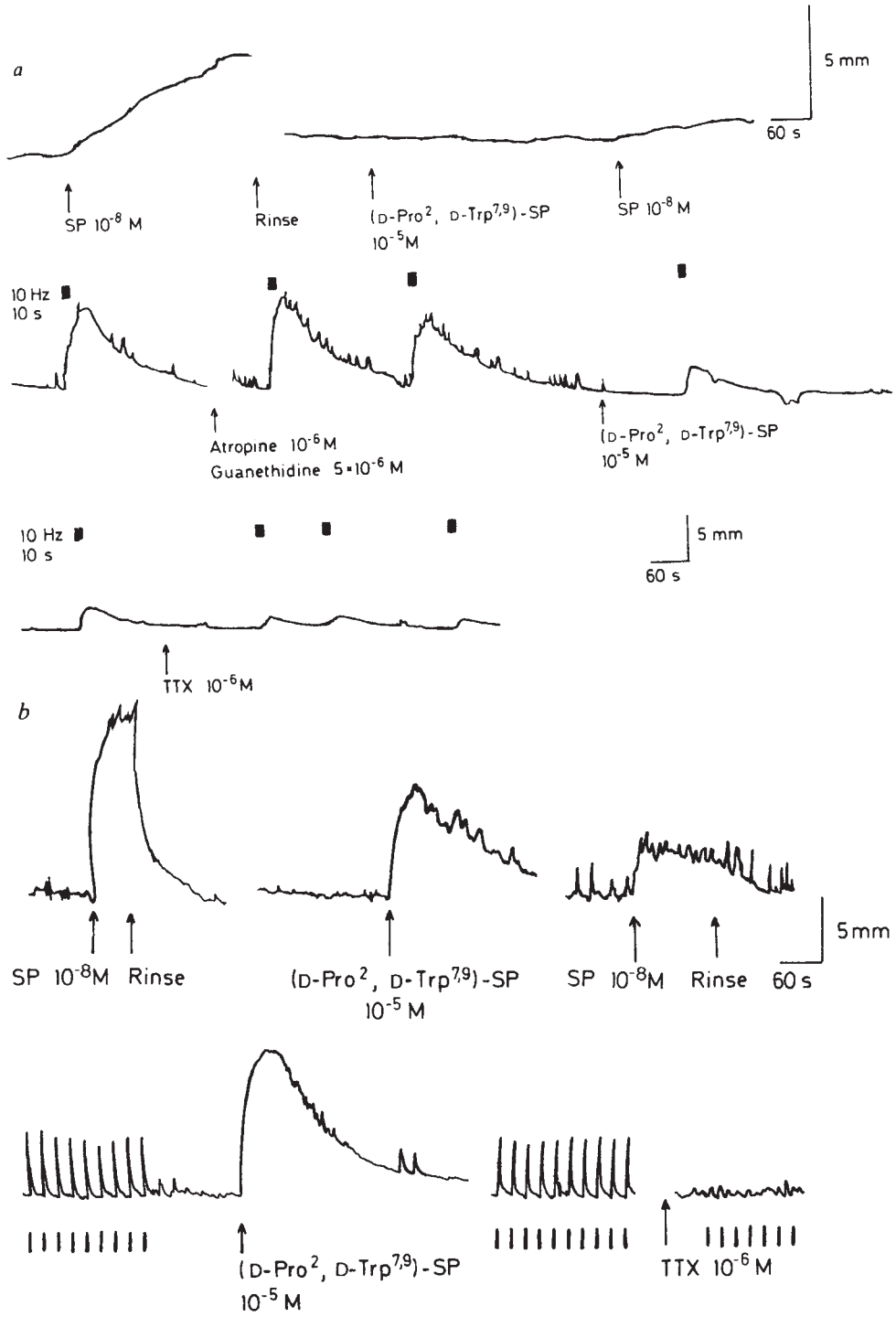

Fig. 4 *a*, Effect of (D-Pro^2, D-Trp^{7,9})-SP on the contractile response to exogenous SP (upper panel) and to the electrically evoked contraction (middle and lower panels) of the rabbit iris sphincter pupillae muscle. In this preparation the SP analogue *per se* had no contractile effect. Electrically induced contraction persisted in the presence of atropine and guanethidine. Note the absence of contractile effect of exogenous SP and the reduction of the electrically evoked contraction following addition of the SP analogue. Addition of tetrodotoxin (TTX; Sankyo) almost completely abolished the residual contractile response. *b*, Effect of (D-Pro^2, D-Trp^{7,9})-SP on the contractile response to exogenous SP (upper panel) and to the electrically evoked contraction (lower panel) of the guinea pig urinary bladder. The muscles were electrically stimulated in the presence of atropine (10^{-6} M) and guanethidine (5×10^{-6} M) with a single pulse every 20 s as indicated. Note the agonist effect of (D-Pro^2, D-Trp^{7,9})-SP, the ensuing blockade of the contractile response to exogenous SP and the lack of effect on the electrically induced contraction on addition of the SP analogue. Electrical stimulation or exogenous SP were applied 5–10 min after the contractile effect of (D-Pro^2, D-Trp^{7,9})-SP had subsided. Addition of TTX completely abolished the contractile response to the electrical field stimulation. The results shown in *a* and *b* are typical examples from a series of four to six experiments.

are probably derived from the trigeminal nerve[15,22,23], which is assumed to have a sensory function. Hence, the SP-mediated contraction is either the result of antidromic nerve stimulation or an indication that the trigeminal nerve carries efferent fibres.

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Distinct subpopulations of enteric p-type neurones contain substance P and vasoactive intestinal polypeptide

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Enteric nerve profiles ultrastructurally distinct from the adrenergic and cholinergic types have been recognized for more than a decade[1]. Baumgarten and his colleagues[2] called these neurones p-type (peptidergic) because they contain large, granular, secretory vesicles similar to those found in peptide-containing nerves in the brain. Subsequently ultrastructural heterogeneity within this extensive non-adrenergic, non-cholinergic p-type system was described, based on the appearance of these granular vesicles[3]. This was consistent with the light microscopical demonstration of increasing numbers of peptides in the gut innervation[4,5], and has been further supported by the localization of vasoactive intestinal polypeptide (VIP) immunoreactivity within large p-type secretory vesicles in the cat proximal colon[6]. Using electron-immunocytochemical techniques on aldehyde-fixed tissue we have shown that the morphological heterogeneity of the p-type system is partly due to the storage of different peptides. We present here evidence that two peptides, VIP and substance P (SP), are present in separate subpopulations of p-type neurones distinguishable by the size and appearance of their granular secretory vesicles. Further subpopulations, unlabelled by specific anti-SP and anti-VIP sera, are also present.

A colloidal gold-labelled second layer antibody was used in a two-step immunocytochemical technique based on the original method of Romano and his colleagues[7]. Purified goat IgG was labelled with colloidal gold particles measuring 20 nm in diameter[8] (see also Fig. 2 legend), in a technique modified from existing methods[7,9-11]. Antisera to SP and VIP were raised in rabbits and characterized for immunocytochemistry (Table 1 and Fig. 2 legend). The colon from adult guinea pigs ($n = 15$) was used (Fig. 1 legend). The method for immunostaining ultrathin sections was similar to that described by Roth *et al.*[12], Larsson[13] and Batten and Hopkins[14]. Briefly, ultrathin sections were etched with 10% H_2O_2 for 15 min, incubated with normal goat serum (1/30 dilution) and then either SP or VIP antiserum for 18 h at 4 °C (Table 1). The sections were rinsed with 0.05 M Tris-buffered saline *p*H 7.3 (TBS) containing 0.2% bovine serum albumin (BSA)[7], before incubation with gold-labelled goat anti-rabbit IgG for 1 h at room temperature (see Fig. 2 legend). Following another wash in TBS/BSA buffer the sections were counterstained with uranyl acetate and lead citrate. Controls were carried out as described in Fig. 2 legend.

Throughout the wall of the guinea pig colon, SP- and VIP-like immunoreactivities were consistently found in the large granular secretory vesicles present in separate and distinct subpopulations of p-type neurones (Fig. 1). Furthermore, these neurones could be distinguished by the size and appearance of the secretory vesicles. Immunoreactivity was observed mainly in

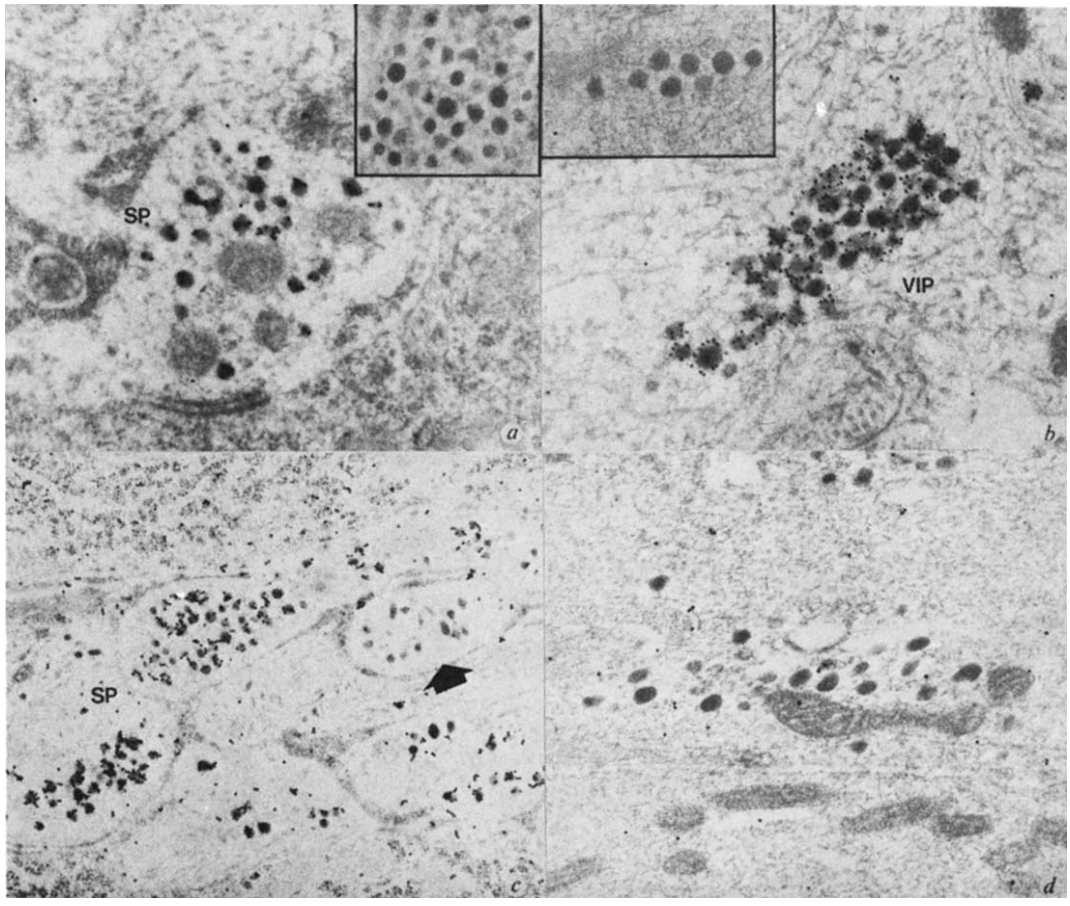

Fig. 1 Ultrathin sections of guinea pig myenteric plexus showing nerve terminals: *a*, immunostained with SP antiserum (SP) (resin polymerized at 60 °C); ×30,000 (inset showing control section stained using SP antiserum preabsorbed with SP antigen (see Table 1); ×30,000). *b*, Immunostained with VIP antiserum (VIP); ×30,000 (inset showing control section stained with VIP antiserum preabsorbed with VIP antigen (see Table 1); ×30,000. *c*, Immunostained with SP antiserum showing positive (SP) and negative (arrow) terminals (resin polymerized at 60 °C); ×20,000. *d*, Immunostained with VIP antiserum (also negative following application of SP antiserum); ×30,000. Note the specific gold labelling over the large, granular, p-type vesicles present in the nerve terminals shown in *a* and *b*. Control sections, as shown in the insets, were completely unstained. The colon was removed from adult Dunkin Hartley guinea pigs under pentobarbitone anaesthesia. Proximal, middle and distal regions were diced and immersion fixed in a cold solution of 3% glutaraldehyde in 0.1 M phosphate buffer *p*H 7.3, for 3 h at 4 °C. The tissue was washed overnight in 0.1 M phosphate buffer *p*H 7.3 containing 0.1 M sucrose, dehydrated and embedded in Araldite. Resin was polymerized either at 60 °C (SP) or at 18 °C using UV light (SP and VIP). Ultrathin sections were cut on a Reichert Ultracut microtome, collected on uncoated 300-mesh nickel grids and immunostained as described in the text. In addition, alternate serial sections from blocks polymerized using UV light were stained with either SP or VIP antiserum so that a direct comparison of vesicle types could be made. A statistical comparison of the results was carried out using an unpaired Student's *t*-test.

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nerve terminals and varicosities although VIP-immunoreactive vesicles were occasionally seen in cell bodies of the submucous plexus (Fig. 2). SP-immunoreactive neurones are also known to be present in the guinea pig gut[4,15] but none was identified in this study. As an extensive study of samples throughout the colon of each animal ($n = 15$) was undertaken, this may be explained either by the presence of a non-reactive precursor form, or a concentration too small to be detected by immunocytochemistry in the cell bodies. Optimal immunostaining of SP and VIP was obtained following different resin polymerization schedules (see Fig. 1 legend). To achieve an accurate comparison of SP- and VIP-immunoreactive vesicles, staining for both peptides was carried out in parallel on alternate serial sections from blocks polymerized at 18 °C with UV light. Section thickness and staining density were standardized as much as possible as these factors are known to affect the electron density of ultrathin sections. Interestingly, the different methods of resin polymerization produced no difference in the size of the vesicles, although they did appear to be slightly less electron dense in the blocks polymerized at the lower temperature. Both SP- and VIP-immunoreactive vesicles were predominantly spherical in shape, although occasionally ovoid or slightly elongated immunoreactive vesicles were observed in terminals of each type. Comparison of the serial sections taken from low temperature cured blocks showed the VIP-immunoreactive vesicles to be significantly larger (mean ± s.d.) (98 ± 19 nm diameter) than the SP-immunoreactive vesicles (85 ± 15 nm diameter) ($P < 0.01$, see Fig. 1 legend), and more electron dense. At least two further subclasses of p-type nerve terminals were present which remained unstained by SP or VIP antiserum. These contained (1) mainly elongated vesicles of medium electron density measuring 74 ± 11 × 91 ± 25 nm (Fig. 1*c*), and (2) large (105 ± 31 nm), spherical vesicles with a homogeneous, dense core (Fig. 1*d*).

We present here evidence that within the heterogeneous p-type innervation of the guinea pig gut, two of the most abundant gut neuropeptides, SP and VIP, are stored in distinct types of nerve terminals distinguishable by the size and appearance of their large, dense-cored secretory vesicles. In addition, further subclasses of p-type terminals, unstained by either SP or VIP, can be seen. The present stage of the classification of peptide-containing nerves is reminiscent of the early description of all gut endocrine cells as enterochromaffin cells[16]. These were subsequently subdivided ultrastructurally[17], but it was not until immunocytochemical techniques were applied that a functional classification could be attempted. This led to the identification of the peptides present in 14 out of the 18 cell types[18]. The original p-type terminals have reached the stage of morphological subclassification[3] and two subtypes have been reclassified by immunocytochemistry. Of the nine types of nerve profiles described, three variations of p-type terminal were reported by Cook and Burnstock[3] according to vesicle size and appearance. The types 5b (see their Fig. 28) and 5c (see their Fig. 29) resembled most clearly the SP- and VIP-immunoreactive vesicles, respectively, as described here. Conclusive identification is difficult because fixation conditions in the two studies were not identical. As a wide range of biologically active peptides is known to be present in the innervation of the mammalian gut[4,5], it is likely that at least some of these will be localized to further kinds of p-type terminal. The availability of immunocytochemical methods for use on ultrathin sections, such as those using colloidal gold[8,12–14], which allow the visualization of a specific immunoreaction with minimal loss of ultrastructural detail, will be invaluable in establishing the full heterogeneity of the gut p-type innervation.

Table 1 Characterization of antisera for immunocytochemistry

Antiserum to	Reference	Titre	Absorption (1–10 nmol per ml diluted antiserum) BN	VIP	SP	GIP	GLUC	SEC
VIP	(652)	1/6,000	+	−(5)	+	+	+	+
SP	(479)	1/8,000	+	+	−(3)	+	+	+

Reference: antiserum reference number. Controls included the application of antiserum preabsorbed with bombesin (BN), gastric inhibitory polypeptide (GIP), glucagon (Gluc), secretin (Sec), VIP and SP; +, no change in the intensity of immunostaining; −(nmol ml^{-1}), minimum amount of peptide required to abolish the immunostaining.

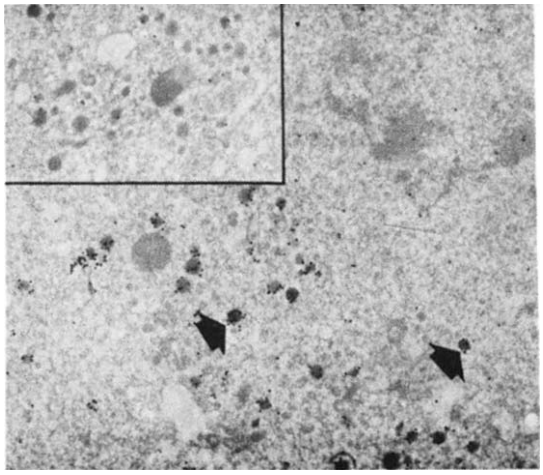

Fig. 2 Ultrathin section of guinea pig submucous plexus showing a VIP-immunoreactive neuronal cell body. Note the specific gold labelling over the large p-type vesicles present in the cytoplasm (arrows); ×20,000 (inset showing serial section stained with VIP antiserum preabsorbed with VIP antigen (see Table 1); ×20,000). SP and VIP antisera were raised in rabbits using synthetic SP (Beckman) coupled to thyroglobulin, and natural porcine VIP (donated by Professor V. Mutt, Karolinska Institute, Stockholm) coupled to haemocyanin with glutaraldehyde, respectively. Both antisera were characterized for immunocytochemistry and found to be directed to the C-terminal portions of their respective molecules. Controls for immunocytochemistry included incubation with the following in place of the first layer antiserum: (1) SP or VIP antiserum preabsorbed with synthetic SP or VIP antigen respectively (1–10 nmol ml^{-1} diluted antiserum, see Table 1); (2) non-immune serum; (3) gold-labelled antibody alone; (4) SP or VIP antiserum preabsorbed with other gut peptides (up to 40 nmol ml^{-1} diluted antiserum). Control sections treated as in (1) to (3) were devoid of specific gold labelling. In control (4) labelling for either SP or VIP was not diminished by the addition of any other gut peptide including bombesin, a peptide known to share two C-terminal amino acids with SP, and glucagon, secretin or gastric inhibitory polypeptide (GIP), which are structurally related to VIP (see Table 1). Gold-labelled goat IgG was prepared[8]. Briefly, purified goat anti-rabbit IgG was dialysed against 2 mM borax buffer *p*H 9.0. Protein aggregates were removed by centrifugation (100,000*g* for 1 h at 4 °C). The optimal amount of antisera necessary to stabilize the gold solution[10] was added dropwise with stirring to the gold solution at room temperature. After 1–2 min stirring a stock solution of bovine serum albumin (BSA) (10% in water) was added to a final concentration of 1%. Unbound proteins were removed by three cycles of centrifugation (14,000*g* for 1 h at 4 °C in 1% BSA/TBS buffer *p*H 8.2 + 2 × 10^{-2} M sodium azide). The last pellet was resuspended to give a final volume 1/10 that of the original gold solution. For immunocytochemistry a dilution of 1/4 was made in TBS containing 0.2% BSA.

SP and VIP antibodies were raised in collaboration with Dr S. R. Bloom (Department of Medicine, Hammersmith Hospital). L.P. is an MRC student. This work was supported by funds from the MRC and Janssen Pharmaceuticals.

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GABA reduces binding of ^{3}H-methyl β-carboline-3-carboxylate to brain benzodiazepine receptors

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Benzodiazepines are thought to exert their pharmacological and clinical effects by interacting with specific receptors on neurones in the central nervous system[1-3]. Originally, only benzodiazepinoid compounds were known to interact with these receptors, but recently other classes of agents have been discovered which have high affinity for benzodiazepine receptors[4]. A representative from one of these classes, ethyl β-carboline-3-carboxylate (β-CCE), was obtained from human urine by virtue of its high affinity for benzodiazepine receptors. It was hypothesized that derivatives or congeners of this β-carboline could be related to presumed endogenous ligands, the exact nature of which are unknown[3,5]. Another ester of β-carboline-3-carboxylic acid, the propyl ester, PrCC, has recently been used as a radioligand for labelling benzodiazepine receptors[6]: in particular, ^{3}H-PrCC has been observed selectively to label a BZ$_1$ receptor subclass[7]. Binding of PrCC to benzodiazepine receptors, however, was less enhanced by γ-aminobutyric acid (GABA) than expected[6,8]. The affinity of benzodiazepines for benzodiazepine receptors is enhanced two to threefold by GABA[9-11], probably reflecting the functional coupling of benzodiazepine receptors and GABA receptors at the molecular level. Here we have investigated binding of the methyl ester of β-carboline-3-carboxylic acid (β-CCM), which by itself is a convulsant, in contrast to β-CCE and PrCC. We report that ^{3}H-β-CCM binds to brain benzodiazepine receptors and that, in contrast to binding of ^{3}H-diazepam, ^{3}H-β-CCM binding is reduced by GABA in a bicuculline-sensitive manner.

^{3}H-β-CCM bound readily to membranes prepared from rat forebrain, hippocampus and cerebellum. Equilibrium was achieved in 10 min at 0 °C and did not change for at least 80 min (data not shown). Specific binding of ^{3}H-β-CCM was defined as the difference between total binding and nonspecific binding, which is binding in the presence of a surplus of unlabelled diazepam (3×10^{-6} M). By using a benzodiazepine instead of a β-carboline to define nonspecific binding, we excluded interference from presumed 'β-carboline sites', unrelated to benzodiazepine receptors. Scatchard analyses of saturation data yielded apparently straight lines (Fig. 1), indicating binding to single classes of sites having affinity constants of 0.4–0.8 nM. The values obtained for K_D are in reasonable agreement with the K_I value = 1.4 nM for inhibition by β-CCM of ^{3}H-flunitrazepam (^{3}H-FNM) binding in rat cerebellum[7].

The number of binding sites for ^{3}H-β-CCM in crude rat cerebellar membranes averaged 56 ± 5 pmol per g tissue (mean ± s.e.m., n = 4) which is only 20% less than the number of ^{3}H-FNM binding sites (71 ± 6 pmol per g tissue; mean ± s.e.m., n = 3). In the rat hippocampus, however, the number of binding sites for ^{3}H-β-CCM (63 ± 2 pmol per g tissue) was 42% less than that of ^{3}H-FNM binding sites (108 ± 4 pmol per g tissue; mean ± s.e.m., n = 3). This is similar to the situation for the radioligand ^{3}H-PrCC, which was interpreted as ^{3}H-PrCC binding preferentially to a BZ$_1$ benzodiazepine receptor subclass[7]. The pharmacological selectivity of ^{3}H-β-CCM binding sites was very similar to that of ^{3}H-diazepam and ^{3}H-PrCC binding sites[1,6]; only those agents that act on benzodiazepine receptors inhibit specific ^{3}H-β-CCM binding and do so at appropriate concentrations (Table 1). These results, together with the chemical similarity of ^{3}H-β-CCM to the more closely examined ^{3}H-PrCC[6,7], suggests that ^{3}H-β-CCM labels brain BZ receptors, probably with preference for the BZ$_1$ receptor subclass.

Our main purpose here was to investigate whether ^{3}H-β-CCM binding was sensitive to GABA receptor stimulation. β-CCM was selected for investigation because it produces pentylenetetrazole-like cloniotonic convulsions in 40–50% of male mice when administered alone in doses which occupy CNS benzodiazepine receptors (10–30 mg per kg intraperitoneally (i.p.), unpublished data). The homologue ester, β-CCE, hardly ever induces convulsions in rats or mice. However, β-CCE enhances the convulsant actions of pentylenetetrazol and bicuculline (a proconvulsant action; refs 12–14 and our unpublished observations). Pharmacological effects of PrCC are not easily determined because very little of this agent reaches the brain after i.p. administration; intravenous administration of 50–200 mg per kg does not produce convulsions in male mice (unpublished results).

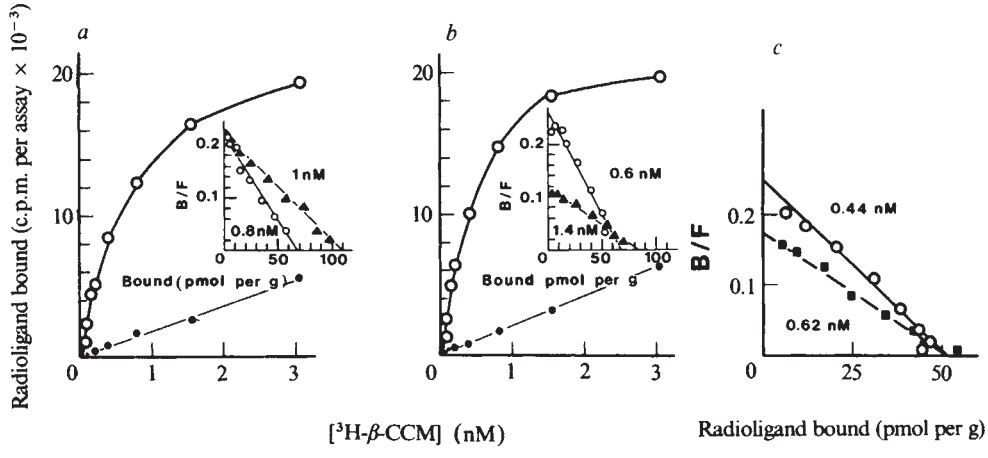

Fig. 1 Saturation of specific binding of ^{3}H-β-CCM and ^{3}H-flunitrazepam (0.06–8 nM) in rat brain membranes. ○, Specific ^{3}H-β-CCM binding; ■, specific binding of ^{3}H-β-CCM in the presence of muscimol (10^{-5} M); ●, nonspecific ^{3}H-β-CCM binding; ▲, specific ^{3}H-flunitrazepam binding. Binding assays were done essentially as described for ^{3}H-PrCC[6]. Crude membranes: hippocampus (*a*) or cerebellum (*b*) were rapidly removed and homogenized in 2 × 10 ml ice-cold KH_2PO_4 (25 mM), *p*H 7.1, for a few seconds in an Ultra-Turrax homogenizer. The homogenate was centrifuged for 15 min at 48,000*g* and the pellet resuspended (500 ml per g of original tissue) in another portion of ice-cold buffer. Duplicate aliquots (2.5 ml) of this crude membrane suspension (~90 mg protein per g original tissue) were incubated together with freshly prepared ^{3}H-β-CCM in water (76 Ci mmol^{-1}; NEN) for 60 min in an ice bath in a total volume of 2.675 ml. Specific binding was obtained by subtracting nonspecific binding, which is binding in the presence of diazepam (final concentration 3×10^{-6} M) from total binding. After incubation the samples were filtered through Whatman GF/C glass fibre filters and washed immediately with 3 × 5 ml of ice-cold assay buffer. Radioactivity on the filters was determined by conventional liquid scintillation counting in mini-vials. ^{3}H-flunitrazepam (85 Ci mmol^{-1}; NEN) binding assays were conducted in a volume of 1.1 ml using 2 mg of original tissue. *c*, Washed membranes. In experiments designed to investigate the effect of GABA on benzodiazepine receptor binding, an extensively washed, unfrozen tissue preparation was used. Brain tissue (100–1,000 mg) was homogenized using an Ultra-Turrax homogenizer in 2 × 10 ml ice-cold 100 mM Tris-citrate, *p*H 7.1, and centrifuged for 10 min at 48,000*g*. The pellet was washed five times in 20 ml Tris-citrate buffer, and each time homogenized and centrifuged as before. The final pellet was resuspended (500 ml per g of original tissue) in Krebs–phosphate buffer as indicated (~50 mg of protein per g original tissue). Binding assays were done as described for crude membranes, except that the incubation time was restricted to 20 min at 0 °C. The Krebs–phosphate buffer contained (final concentration, mM) NaCl (122), KCl (4.8), $CaCl_2$ (0.97), $MgSO_4$, $7H_2O$ (1.2) and Na_2HPO_4 (16). Values in *a*, *b* and *c* are from three single experiments, which were all repeated with similar results. Respective K_D values are shown. B/F, the proportion bound to free radioligand, is expressed as c.p.m. per assay/c.p.m. per assay.

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We found that specific binding of ^{3}H-β-CCM to rat cerebral cortex membranes was reduced in the presence of the GABA agonist muscimol, a finding in clear contrast to the enhancement of ^{3}H-diazepam binding (Table 2). Muscimol, GABA and the anomalous GABA agonist piperidine-4-sulphonic acid[11], similarly reduced specific binding of ^{3}H-β-CCM in rat cerebellar membranes. The effects of muscimol and GABA, and to a lesser extent of piperidine-4-sulphonic acid (data not shown), were reduced by bicuculline (Table 3). Bicuculline is believed to be a specific GABA antagonist and the results thus indicate that GABA and muscimol reduce ^{3}H-β-CCM binding at least partially by an effect on the GABA receptors. The affinity rather than the number of ^{3}H-β-CCM binding sites was reduced by muscimol (Fig. 1*c*). β-CCM did not inhibit high-affinity ^{3}H-muscimol binding to presumed GABA receptors (unpublished results).

Table 1 Inhibition of specific ^{3}H-β-CCM binding in rat cerebellum

Inhibitor	IC_{50} (nM)
Lorazepam	1
β-CCE	1.5
PrCC	1.5
β-CCM	3
Diazepam	20
CL 218872	50
Norharman	2,000
Ro 5-4864	13,000
Ro 5-3663	200,000
Ethanol	$\sim 2\times10^6$
Meprobamate	>30,000
Pentobarbital	>30,000
Diphenylhydantoin	>30,000
Theophylline	>30,000
Strychnine	>30,000
Picrotoxin	>30,000
Pentylenetetrazole	>30,000
Methysergide	>30,000

Crude membranes were used (see Fig. 1 legend) at a concentration of 5 mg original tissue per 2.675 ml of assay; control specific binding was 6,100 ± 250 c.p.m. per assay (mean ± s.e.m., $n = 5$), nonspecific binding was 600 ± 40 c.p.m. per assay (mean ± s.e.m., $n = 5$). ^{3}H-β-CCM was used at a concentration of 0.35 nM, specific activity 76 Ci mmol^{-1} (NEN). Each IC_{50} value shown is the mean of two independent determinations, each obtained by adding five to six concentrations of the agent to duplicate assays. The concentration causing 50% inhibition of specific binding (IC_{50} value) was determined graphically. By pairing the two determinations obtained for each of the nine inhibitory agents, the standard deviation was estimated as 25% of the mean value.

Table 2 Effects of muscimol on specific radioligand binding to rat cerebral cortex

Radioligand	Concentration (nM)	Nonspecific binding (%)	Specific binding (c.p.m. per assay) Without muscimol	With muscimol (10^{-5} M)	%
^{3}H-diazepam	0.34	21	517 ± 3	1,087 ± 7	210*
^{3}H-PrCC	0.35	29	2,712 ± 210	3,430 ± 62	126†
^{3}H-β-CCE	0.29	12	1,686 ± 130	1,534 ± 19	91
^{3}H-β-CCM	0.32	17	902 ± 6	746 ± 2	83*

Tris-citrate buffer was used both for washings and assays; for further details see Fig. 1 legend. Nonspecific binding is shown as a % of total binding in the group 'without muscimol'. Absolute values for nonspecific binding in the group 'with muscimol' were indistinguishable from those in the group 'without muscimol'. ^{3}H-PrCC is ^{3}H-propyl β-carboline-3-carboxylate (40 Ci mmol^{-1}; A/S Ferrosan). Lower values for nonspecific binding were obtained using fresh batches of ^{3}H-PrCC, for example, NET 703 (NEN). ^{3}H-β-CCE is ethyl β-[6(n)-^{3}H]carboline-3-carboxylate (24.6 Ci mmol^{-1}; Amersham). ^{3}H-β-CCM is methyl β-[6(n)-^{3}H]carboline-3-carboxylate (24.6 Ci mmol^{-1}). Values shown are for a single experiment, which was repeated with similar results. Specific binding is given as mean ± s.e.m. of three values.

* $P < 0.001$; † $P < 0.05$.

Table 3 Muscimol and GABA reduce specific cerebellar binding of ^{3}H-β-CCM; antagonism by bicuculline

Expt	Addition	Specific binding of ^{3}H-β-CCM (c.p.m. per assay; mean ± s.e.m. (n))	% Of control
	Vehicle (control)	3,225 ± 57 (8)	100
	Muscimol (10^{-6} M)	2,305 ± 124 (4)	71*
	Muscimol (10^{-6} M) + bicuculline 10^{-4} M	2,673 ± 110 (4)	83†
(1)	Muscimol (3×10^{-6} M)	2,501 ± 61 (4)	78*
	Muscimol (3×10^{-6} M) + bicuculline (10^{-4} M)	2,979 ± 59 (4)	92†
	Bicuculline (10^{-4} M)	3,063 ± 174 (4)	95
	Piperidine-4-sulphonic acid (10^{-4} M)	1,773 ± 40 (4)	55*
	Vehicle (control)	5,656 ± 89 (6)	100
	GABA (10^{-6} M)	4,770 ± 50 (3)	84‡
	GABA (10^{-6} M) + bicuculline (10^{-4} M)	5,520 ± 110 (3)	98§
	GABA (3×10^{-6} M)	4,700 ± 20 (3)	83*
(2)	GABA (3×10^{-6} M) + bicuculline (10^{-4} M)	5,210 ± 40 (3)	93‖
	GABA (10^{-5} M)	4,350 ± 50 (3)	77*
	GABA (10^{-5} M) + bicuculline (10^{-4} M)	4,880 ± 30 (3)	87‖
	GABA (10^{-4} M)	4,510 ± 30 (3)	80*
	GABA (10^{-4} M) + bicuculline (10^{-4} M)	4,060 ± 90 (3)	73§
	Bicuculline (10^{-4} M)	5,606 ± 113 (6)	99

Washed rat cerebellar membranes were resuspended in Krebs-phosphate buffer for assays using 0.37 nM ^{3}H-β-CCM (76 Ci mmol^{-1}) as described in Fig. 1 legend. The amounts of original tissue used in experiments 1 and 2 were 5 and 8 mg, respectively, per 2.675 ml of assay. Nonspecific binding in the presence of diazepam (3×10^{-6} M) was 14% of total binding.

* $P < 0.001$; ‡ $P < 0.01$ compared with control (Student's t-test). † $P < 0.01$; § $P < 0.04$; ‖ $P < 0.001$ compared with GABA or muscimol alone (Student's t-test).

In another series of experiments we investigated the effect of muscimol on inhibition of specific ^{3}H-β-CCM binding by flunitrazepam. The 50% inhibitory concentration (IC_{50}) for flunitrazepam was 5.9 nM in the absence of muscimol, compared with 2.7 nM in the presence of muscimol (10^{-5} M) (washed rat cerebellar membranes were used, as described in Fig. 1 legend). On the other hand, inhibition of ^{3}H-β-CCM binding by unlabelled β-CCM was slightly reduced by muscimol (IC_{50} of 1.3 and 1.7 nM, respectively, in the absence and presence of muscimol (10^{-5} M)). This experiment shows that those benzodiazepine receptors to which ^{3}H-β-CCM bind increase their affinity for a benzodiazepine by GABA receptor stimulation and that ^{3}H-β-CCM binding sites are indeed coupled to GABA receptors.

Our results are in agreement with the notion that β-carboline-3-carboxylic acid esters bind to benzodiazepine receptors (presumably the BZ_1 receptor subclass) and that these benzodiazepine receptors are coupled to GABA receptors. The conformational change in benzodiazepine receptors induced by GABA, however, affects the binding affinity of a convulsive β-carboline in a manner exactly opposite to the affinity of benzodiazepines. Thus the convulsive β-carboline and anticonvulsant benzodiazepines recognize benzodiazepine receptors in different ways. It is tempting to speculate that the sensitivity to GABA reflects 'agonist/antagonist' potentials of benzodiazepine receptor ligands (see also ref. 8). Furthermore, an intermediate class of ligands may exist, the binding of which is mainly unaffected by GABA and which lack obvious pharmacological effects when given alone. The arguments presented here rely mainly on observations made with β-CCM; further studies are needed, ideally including more efficient and biologically stable convulsive ligands.

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ACh release from osmotically shocked synaptosomes refilled with transmitter

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It is still uncertain whether or not acetylcholine (ACh) released at the synapse is derived from vesicles. We have recently described a sensitive chemiluminescent technique which allows continuous measurement of ACh release from tissue slices or synaptosomes incubated in the reaction mixture[1,2]. From the luminescence obtained when synaptosomes are opened in the reaction mixture by a single freezing and thawing we estimated that 30% of the total ACh of *Torpedo* electric organ synaptosomes is cytoplasmic. The vesicular ACh compartment which resists freezing and thawing requires a detergent to be liberated. These estimations showed that the ACh compartment considered as cytoplasmic was readily released from stimulated synaptosomes[3]. If we are dealing with a real, soluble cytoplasmic ACh compartment, it should in principle be possible to recover, in controlled ionic conditions, a calcium-dependent ACh release from synaptosomes emptied by an osmotic shock, and refilled at the desired ACh concentration. We show here that this is the case. These results suggest the possibility of a non-vesicular release of ACh from the nerve terminal.

Torpedo electric organ synaptosomes were prepared[4,5] and concentrated in a physiological solution of 1.1 osmol. After inhibiting acetylcholinesterase using phospholine (0.8×10^{-3} for 30 min), the synaptosomes were given an osmotic shock in a large volume of hypotonic solution containing 25 mM K_2SO_4, 10 mM Tris-HCl *p*H 6.9, 0.3 mm ATP, 0.3 mM $MgCl_2$ and a range of ACh concentrations (0–40 mM). Glycine was substituted for ACh at the lower ACh concentrations. After 30 min, the shock period was ended by adding a KCl solution (300 mM final concentration) which also contained ACh and glycine to maintain the concentrations of these substances. After 30 min, the refilled synaptosomal sacs, equilibrated with ACh and KCl, were centrifuged (27,000*g*, 30 min), resuspended and sedimented again, before placing them in an 'external medium' consisting of 300 mM NaCl, 25 mM Na_2SO_4 and 10 mM Tris-HCl *p*H 8.6. (The alkaline *p*H necessary for the luminescent reaction did not affect ACh release, as we had previously ensured that the electrical discharge of the tissue remained constant for 3 h at that *p*H[2].) The refilled synaptosomal sacs were resuspended in 100 μl of external medium; 10 or 20 μl were used per assay (this gives ~1 nmol entrapped ACh when the sacs are filled with 40 mM ACh). Considering that these sacs derive from 400 mg of tissue, and that the initial synaptosomal ACh content was ~15 nmol, the yield of this procedure is ~6%.

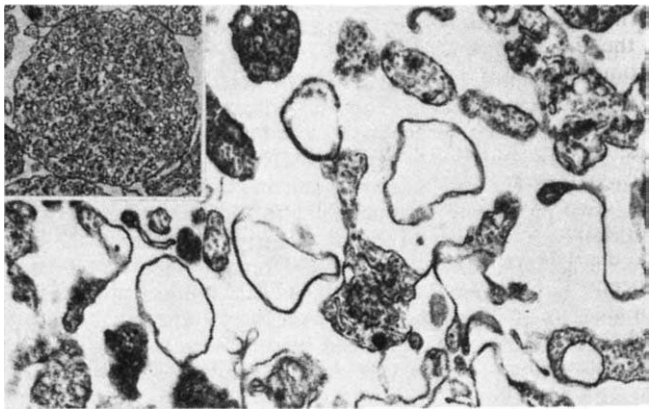

Fig. 1 Synaptosomal sacs. The sacs are either empty or contain glycogen-like granules. Synaptic vesicles are lost during preparation. ×30,800. The insert shows for comparison a synaptosome before shock. Note the numerous synaptic vesicles and the difference in size. ×10,450.

Table 1 Ionophore plus Ca^{2+} required for ACh release

	ACh release (amplitude of trace in pmol)
Ionophore A23187 (without Ca^{2+})	1.25 ± 0.42 (5)
Ca^{2+} (without ionophore A23187)	3.00 ± 0.5 (3)
Ionophore A23187 + Ca^{2+}	11.2 ± 2.7 (12)

The ACh release induced by ionophore + Ca^{2+} is significantly different from that in non-release conditions, in which either calcium or ionophore are omitted: $0.01<P<0.001$. No. of determinations is shown in parentheses.

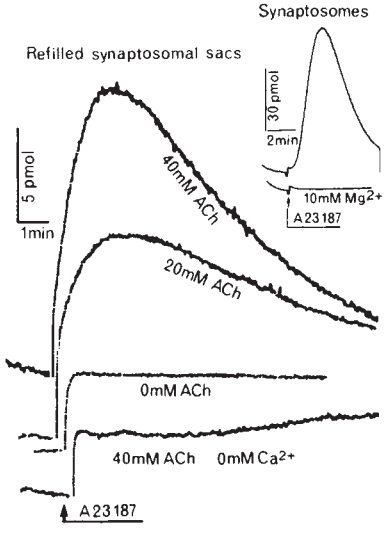

Fig. 2 ACh release from refilled synaptosomal sacs. The synaptosomes were osmotically shocked at 20 °C and refilled with a range of ACh concentrations. The release of ACh triggered by the calcium ionophore A23187 (3.8 μM) increased in proportion to the indicated ACh concentration used to refill the sacs. The omission of calcium greatly reduced ACh release. The insert shows the release of ACh from intact synaptosomes containing ~1 nmol ACh (similar to that from synaptosomal sacs filled with 40 mM ACh). The release from synaptosomes was much reduced (lower trace) when magnesium replaced calcium. The release curve for synaptosomes is comparable to that for refilled synaptosomal sacs. The reaction mixture consisted of 470 μl external medium, with or without 10 mM $CaCl_2$, 2.6 units choline oxidase (Wako), 10 μg horseradish peroxidase type II (Sigma), 10 μl luminol (1 mM; Merck) and 1.7 units acetylcholinesterase from *Electrophorus* (Boehringer) passed through Sephadex G-50.

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The amount of entrapped ACh was, as expected, proportional to the ACh concentration used to refill the shocked synaptosomes. This was verified by disrupting the sacs in the reaction mixture with Triton X-100. Most (85%) of the entrapped ACh was liberated by freezing and thawing the refilled synaptosomal sacs, in contrast to intact synaptosomes where most of the ACh (70%) is bound to synaptic vesicles, resists freezing and thawing and requires a detergent or an osmotic shock to be liberated. A morphological analysis of the refilled synaptosomal sacs showed that the synaptosomes had indeed lost their structure during the procedure (see Fig. 1). The sacs lost their synaptic veiscles but not all were empty as some retained a reticulum-like network and glycogen-like granular material.

Substantial ACh release from the refilled synaptosomal sacs was observed after calcium influx had been triggered by the calcium ionophore A 23187. This release of ACh increased in proportion to the internal ACh concentration (Figs 2, 3*a*). For comparison, the release of ACh from intact synaptosomes containing ~1 nmol ACh (similar to the sample of sacs filled with 40 mM ACh) is shown in Fig. 2 insert. For both cases the release curves were very similar. To measure the proportion of ACh content of the sac that was released as a result of the calcium influx, we estimated ACh content before and after triggering the release by disrupting the sacs with Triton X-100. Their ACh content declined by as much as 50% in 10 min, at which time the release curve still represents 25% of its maximum amplitude. The specificity for ACh of the release process was tested by filling the sacs with choline. In this case there was a 70% reduction in release, indicating a clear preference for ACh (see Fig. 3*a*). The calcium dependency of transmitter release from synaptosomal sacs refilled with 40 mM ACh is shown in Fig. 2 (lower trace). There was no release when ionophore was added in the absence of calcium. When extracellular calcium was increased, the ionophore became efficient (Fig. 2, upper trace). The amplitude of ACh release triggered by the ionophore clearly increased when the extracellular calcium concentration was increased (Fig. 3*b*).

Gramicidin D, which triggers ACh release from intact synaptosomes[3], also acted on the synaptosomal sacs, which released ACh in proportion to their content. In both cases the release of ACh was much depressed when magnesium was substituted for calcium.

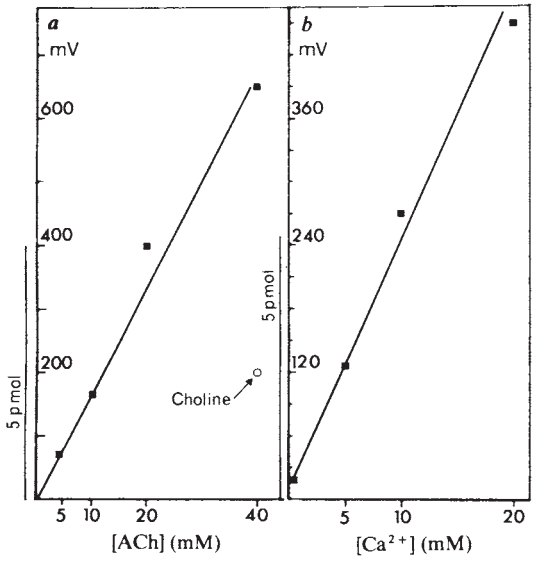

Fig. 3. *a*, Effect of internal ACh concentration on its release from synaptosomal sacs. The amplitude of ACh release curves is plotted for the various ACh concentrations used to refill the shocked synaptosomes. ACh release was triggered by the calcium ionophore as in Fig. 2. Note that when choline was substituted for ACh (o), release was much reduced. *b*, Effect of external calcium on amplitude of ACh release. Synaptosomal sacs filled with 40 mM ACh were treated with ionophore A23187 at various extracellular calcium concentrations. The amplitude of release curves increased in proportion to extracellular calcium concentration.

To determine whether the *p*H gradient (acid in the sac) was involved in the observed ACh release, we repeated the experiment shown in Fig. 2 at a constant *p*H (7.9) inside and outside the sac. When ionophore A23187 was added in the absence of calcium, there was no significant ACh release but this was triggered by the subsequent addition of calcium. If calcium was added in the absence of ionophore, only a small amount of ACh was released and the subsequent addition of ionophore A23187 triggered the usual ACh release (Fig. 2). Table 1 gives the mean amplitude of the ACh release curve compared with that for non-release conditions. It is clear that ACh release from the sacs is calcium dependent, even at constant *p*H.

In conclusion, we were able to obtain a calcium-dependent ACh release from synaptosomal membrane sacs filled with ACh in controlled ionic conditions. The release of ACh increased in proportion to the internal ACh content and to the external calcium concentrations. This model might serve as a means of studying release of cytoplasmic ACh recently measured for intact synaptosomes[3]. We do not exclude the possibility that a vesicular mechanism coexists with the one described here, which cannot be attributed to synaptic vesicles. The intramembrane particles of the presynaptic membrane, which undergo important changes during synaptic activity[6,7], may play a prominent part in release of cytoplasmic ACh.

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Persistent behavioural effect of apomorphine in 6-hydroxydopamine-lesioned rats

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The rotational (circling) behaviour of rodents with unilateral nigrostriatal damage has been used extensively to investigate nigrostriatal function and the action of dopaminergic compounds[1]. Rats lesioned by unilateral microinjection of the catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), spontaneously exhibit slight ipsilateral (towards the lesioned side) rotation. Systemic challenge with the dopamine receptor agonist apomorphine induces active contralateral rotation, apparently as a result of supersensitivity in the lesioned hemisphere[2,3]. We report here that apomorphine treatment also results in an extraordinarily persistent change in spontaneous (undrugged) rotation. After one treatment with 50 μg per kg apomorphine, exposure to the rotation environment resulted in a brief, intense burst of contralateral rotation. This behaviour, apparent for months after drug treatment, did not fully develop until 2 weeks after treatment and was never observed in lesioned, but otherwise drug-naive rats. The latent apomorphine effect, unlike the acute effect, was inversely related to dose over the range tested. The behaviour was relatively specific for the environment in which drug treatment had occurred, suggesting a role for learning, but it is difficult to reconcile the inverse dose–response function, lack of recency effect and inability to induce an analogous phenomenon using (+)amphetamine with this explanation.

Male Sprague–Dawley rats (180–240 g) were lesioned unilaterally by injection of 8 μg (base) 6-OHDA hydrobromide (Aldrich). Two to four weeks later rats were injected subcutaneously (s.c.) with saline or apomorphine HCl (0.05, 0.25 or

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1.0 mg per kg; Merck). Each rat was placed in a clear plastic hemispherical bowl (26 cm diameter) and observed for 3-min periods before injection, immediately after injection, and periodically thereafter until rotation had slowed to near zero. Total 360° turns in each direction were counted during each observation period. Net contralateral turns were calculated by subtracting the number of ipsilateral turns from the number of contralateral turns. Rats which made fewer than 10 net contralateral turns in one of the observation periods after apomorphine treatment were considered to be only poorly lesioned (<15% of subjects) and their data were disregarded.

Before injection, all groups exhibited slight ipsilateral bias (Fig. 1*a*). Shortly after apomorphine injection, rapid contralateral rotation began, in the virtual absence of ipsilateral turning. Consistent with previous reports[2,4], this acute effect of apomorphine was dose-related with respect to duration but dose-independent with respect to peak rate. When placed in the bowl 2 weeks after treatment, in a 3-min observation period rats that had received saline continued to show slight ipsilateral bias whereas apomorphine-treated rats showed almost exclusive contralateral rotation, which was inversely related to the previously administered dose (Fig. 1*b*). In the case of the group receiving the lowest dose, the rate of rotation in the 3-min observation period 2 weeks after drug treatment was, remarkably, higher than the peak rate observed immediately after drug administration. The animals rotated in tight circles, most rapidly in the first minute, and soon slowed, usually stopping by the end of the 3-min test; no ipsilateral turns were made. Some of the rats treated with saline, 0.05 or 1.0 mg per kg apomorphine were repeatedly tested for rotation at 2–8-week intervals with no further drug treatment. Behaviour of these subgroups was consistent with that shown in Fig. 1*b* for as long as it was tested: saline-treated rats continued to exhibit slight ipsilateral bias, and rats treated with 0.05 mg per kg apomorphine continued to exhibit rapid contralateral rotation, up to 9 months after treatment. Rats treated with 1.0 mg per kg apomorphine continued to exhibit only slight contralateral rotation up to 14 weeks after treatment.

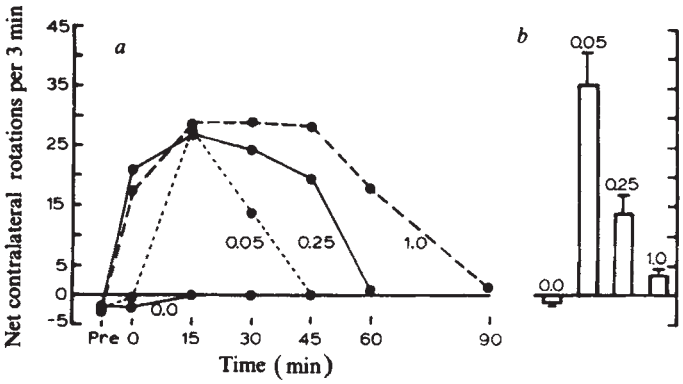

Fig. 1 Acute and long-term rotational response to saline or apomorphine (0.05, 0.25 or 1.0 mg per kg) in 6-OHDA-lesioned rats. 6-OHDA (8 μg base in 4 μl saline and 0.1% ascorbic acid) was injected at a rate of 0.33 μl min^{-1} into 180–240-g rats anaesthetized with pentobarbital. Coordinates (with incisor and ear bars levelled) were 4.2 mm posterior and 1.4 mm lateral from bregma, 8.0 mm deep from skull surface. A single s.c. injection of apomorphine HCl or saline was administered 2–4 weeks post-lesion (n = 10–14 per group). *a*, Mean net contralateral rotations made in 3-min observation periods before injection (Pre), and at the indicated times after injection of saline or apomorphine. *b*, Mean +s.e.m. net contralateral rotations made 2 weeks after saline or apomorphine treatment when rats were placed in the rotation bowl for a 3-min observational period. All groups except the saline-treated showed a significant difference in rotation 2 weeks after treatment compared with spontaneous rotation before treatment (paired Student's t-tests, $P < 0.001$ in each case). The effect of each dose of apomorphine measured 2 weeks after treatment was significantly different from the effect of each of the other doses (one-way analysis of variance followed by Student–Newman–Keuls test, $P < 0.05$ in each case).

Table 1 Persistent rotational response to apomorphine HCl (0.05 mg per kg) in 6-OHDA-lesioned rats as a function of time interval between drug administration and re-exposure to rotation environment

No. of animals	Acute rotational response*	Interval between injection and test (days)	Latent rotational response†
11	28.3±4.0	1	9.5±3.0
8	27.2±3.9	4	11.4±4.1
7	27.8±1.9	7	12.0±2.1
7	27.1±2.5	14	37.8±7.1‡
9	26.9±3.0	28	33.7±7.5‡

* Contralateral rotations per 3 min beginning 15 min post-injection (no ipsilateral turns were made).

† Net contralateral rotations per 3 min on the days indicated (all groups averaged <1 ipsilateral turn).

‡ Significantly different from 1, 4 and 7 day groups ($P < 0.025$; one-way analysis of variance and Student–Newman–Keuls test).

An additional group of lesioned rats (n = 5) was injected with saline before being placed in the bowl for 48 min; after 2 h they were injected with 0.05 mg per kg apomorphine and observed to rotate in their home cages. This group failed to exhibit contralateral rotation when tested in the bowl from 2 to 24 weeks after treatment. Some of these subjects occasionally showed sudden contralateral rotation when returned from the bowl to their home cages. This stimulus (environmental) specificity was tested further in two groups of lesioned rats. Two weeks post-lesion, rats were treated with 0.05 mg per kg apomorphine on three consecutive days. After each injection, subjects were placed in the bowl (n = 5) or in a rectangular (20×21×24 cm) opaque plastic box (n = 5) for 48 min. Two weeks after the last drug treatment, rats that had rotated in the bowl were tested for 3 min in the box and, 30 min later, for 3 min in the bowl. Conversely, rats that had rotated in the box were tested first in the bowl, then in the box. Rats showed significantly greater rotation in the environment in which they had previously received drug treatment than in the novel environment (mean = 36.6±9.6 to 3.3±2.3 net contralateral rotations per 3 min; $P < 0.02$; paired Student's t-test).

As the stimulus specificity of the behaviour suggested a role for learning, five groups of lesioned rats were tested for recency effect. Two weeks post-lesion, rats were treated once with 0.05 mg per kg apomorphine and were observed to rotate in the bowl as described for the dose-response experiment. Rats were then placed in the bowls for a 3-min observation period 1, 4, 7, 14 or 28 days after drug treatment. Each rat was thus re-exposed to the rotation bowl only once after drug treatment. Groups tested 14 or 28 days after drug treatment showed significantly greater contralateral rotation than those tested at *shorter* intervals after treatment (Table 1).

Ungerstedt[5,6] reported that some 6-OHDA-lesioned rats developed 'explosive' contralateral rotation at ~2 weeks post-lesion. As the aetiology was unknown, this was termed 'paradoxical' rotation. We consider that paradoxical rotation and the latent, persistent effect of apomorphine shown here are, in fact, the same phenomenon. We have never observed paradoxical rotation in over 200 lesioned, but otherwise drug-naive, rats at 2 to 30 weeks post-lesion. In rats that had been lesioned 30 weeks previously, it was still possible to induce paradoxical rotation by apomorphine treatment.

The stimulus specificity of paradoxical rotation suggests that it may be an extraordinary example of associative learning. If so, it is difficult to explain the inverse dose relationship and lack of recency effect. Furthermore, associative learning alone would not predict any pharmacological specificity, that is, any compound inducing acute rotation, regardless of direction, might be expected to induce a similar latent, persistent rotation. LSD, which, like apomorphine, induces acute contralateral rotation[7], also resulted in paradoxical rotation after a single treatment (unpublished data). However, we have been unable to induce an analogous phenomenon with either single or repeated

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(+)amphetamine treatments, which induced acute ipsilateral rotation.

Just as associative learning does not seem to fully explain our data, we know no previously described effect of apomorphine that would. The effects of low doses of apomorphine have been associated with presynaptic activity[8-10]. While apomorphine-induced rotation in 6-OHDA-lesioned rats has usually been attributed to direct action on supersensitive striatal neurones, a role in this behaviour has recently been ascribed to drug action in the substantia nigra itself[11]. It may be that in rats having nigrostriatal 6-OHDA lesions, the presynaptic effect of a small dose of apomorphine is greatly exaggerated over that in intact rats. Paradoxical rotation may then be the result of both learning and a persistent biochemical consequence of drug administration.

Our finding that paradoxical rotation results from a single treatment with apomorphine should serve as a caveat to those who routinely verify lesions behaviourally by administering a 'screening' dose of apomorphine. Paradoxical rotation may, however, prove useful for studying chronic effects of acute phychoactive drug administration, for example, phenomena such as LSD flashbacks[12].

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Multiple tubulin forms are expressed by a single neurone

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Microtubules are a key cytoskeletal constituent of the eukaryotic cell, being involved in mitosis, cell division, cell shape, intracellular transport and motility[1]. They are composed of tubulin, a 110,000-molecular weight (M_r) protein which is a dimer of 55,000-M_r α and β submits[2]. Multiple forms of both α- and β-tubulin have been demonstrated biochemically by gel electrophoresis[3,4], column chromatography[5] and peptide maps[6]; immunologically by monoclonal antibodies[7]; and genetically by mapping multiple tubulin genes in the eukaryotic genome[8-10]. There is evidence for multiple tubulin mRNA species[11,12,13], but some of the microheterogeneity may also arise from post-translational modifications such as phosphorylation[14] and glycosylation[15]. Tubulin microheterogeneity is most dramatic in the brain where it could be the result of cellular heterogeneity or a multiplicity of specific functions within each cell, such as neurite extension and axoplasmic transport[1,16]. Functional specificity has been demonstrated in particular tubulin subunits in other tissues[9,16,17]. To determine whether multiple forms of tubulin coexist in single cells, we have purified and characterized the tubulin of single, isolated sympathetic neurones grown in primary cell culture[18]. We show here for the first time that tubulin can be isolated and characterized from a single mammalian neurone. Single neurones exhibit multiple tubulin isoelectric forms, suggesting that structurally different forms of microtubules have functionally different roles in a single cell.

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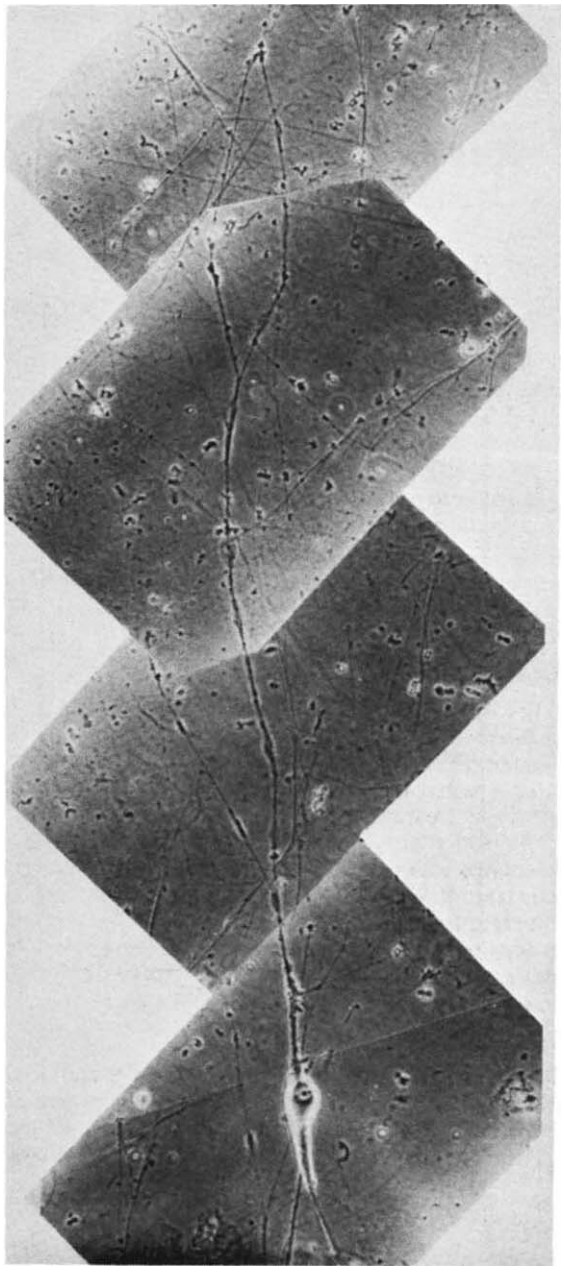

Fig. 1 An isolated neurone grown in a well of a Terasaki plate, viewed by phase-contrast microscopy. Scale bar, 40 μm. Neurones from the superior cervical ganglia of newborn rats were grown as described previously[27]. Briefly, the ganglia were mechanically dissociated and the cells plated on to collagen in a medium containing nerve growth factor (NGF). Cultures were kept in a 5% CO_2 atmosphere in medium containing 20mM K^+. For studies of solitary neurones, Falcon Terasaki tissue culture plates were prepared as follows. The bottom of each well was coated with 1 μl of solubilized collagen and the dish exposed to ammonia vapours. The resulting collagen gels provide a more adhesive surface than dried films of collagen[27]. Medium (10 μl) was added to each well, and the dish was sterilized by exposure to UV light. Ganglia were dissociated first with forceps, passed 10 times through a 23G hypodermic needle, then seeded into the dish (< 100 cells) in 6 ml of culture medium. In successful platings, each plate had three or four wells with isolated neurones (out of a total of 60 wells), the rest of the wells containing none or only small clumps of neurones. Medium was changed every 3 or 4 days.

Single neurones grown in microwells of Terasaki plates showed extensive morphological differentiation after 3 weeks in culture. Figure 1 shows a phase-contrast micrograph of a single cell in an isolated well. The cell has well-defined dendritic and

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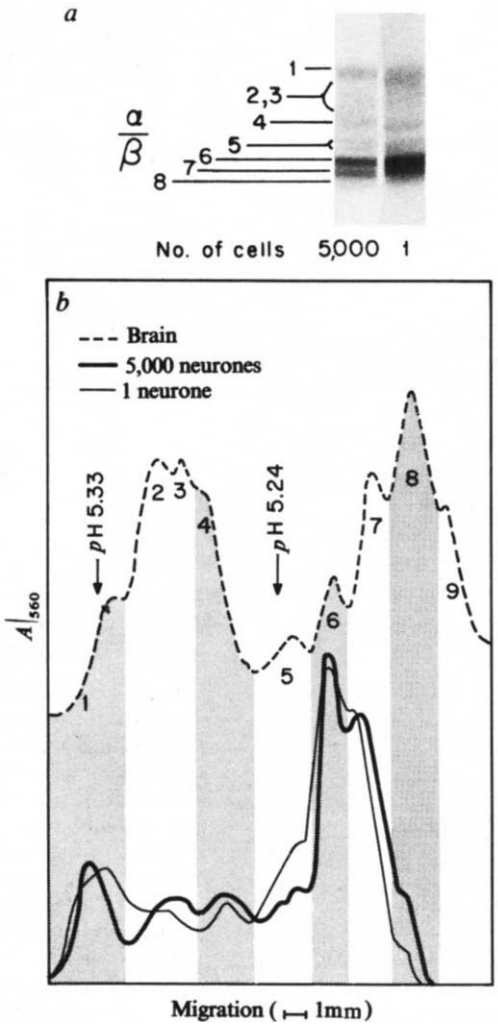
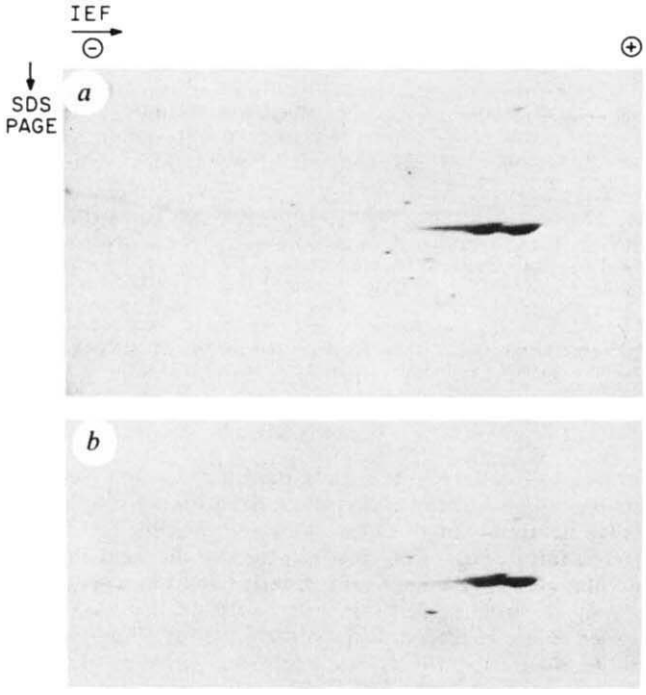


Fig. 2 Multiple tubulin forms are found in a single neurone. Mature cells (3–4 weeks in culture) were labelled with ^{35}S-methionine (900–1, 000 Ci mmol^{-1}, 6–8 mCi ml^{-1}; Amersham) as follows. Mass cultures of 2,000–5,000 cells were incubated for 18 h in 40 μl of methionine-deficient L-15 culture medium (Gibco) containing 10 μM cold methionine and 1 μM ^{35}S-methionine. Cultures were collected and sonicated for 3 min in a bath sonicator (Laboratory Supplies, New York) in the presence of 250 μl of 0.24 M sucrose, 10 mM Na-phosphate buffer *p*H 7.4, 10 mM $MgCl_2$, 0.2 mM GTP and 100 μl of brain homogenate prepared from mature rat brain in 2 vol of the same buffer. The resulting homogenates were centrifuged first at 12,000 *g* for 10 min and then at 100,000 g for 1 h. Vinblastine sulphate (1 mg ml^{-1}) was added to the high-speed supernatants. Samples were incubated at 37 °C for 30 min, then the tubulin aggregates were sedimented at 5,000 g for 5 min. The tubulin-enriched pellets[28] were each suspended in 50 μl of lysis buffer[29] and 10-μl aliquots were subjected to isoelectric focusing[29]. Single cells in microwells of Terasaki tissue culture plates were incubated in 10 μl of methionine-deficient medium containing 10 μM ^{35}S-methionine for 18 h. They were then collected in 50 μl of the buffer used for the mass cultures plus 0.5% Triton X-100 and were sonicated in the presence of 20 μl of brain homogenate. (The addition of Triton X-100 did not interfere with subsequent experimental procedures.) Vinblastine precipitation and isoelectric focusing were performed as above, except that all the material obtained from a single cell was applied to one isoelectric focusing gel. The final *p*H gradient was determined by eluting 2-mm portions of a blank gel in 10ml H_2O. Gels were stained, dried and exposed to Kodak X-R5 X-ray film and Dupont Cronex lighting-plus intensifying screens[30] for 1 to 3 weeks, depending on the amount of label present. The numbers identifying the various isotubulin bands were assigned by alignment with Coomassie brilliant blue staining of the rat brain tubulin carrier. *a*, Autoradiogram of the isoelectric focusing gels; *b*, densitometric tracings of the autoradiogram shown in *a*. Shaded areas were divided according to the peaks of the isotubulin bands of the sympathetic neurones and aligned with the co-migrating brain carrier. Nine isotubulins are observed in the brain tubulin; 1–4 comprise the classically defined α-tubulin subunit, and 5–9 the β subunit[3]. The co-electrophoresed cold carrier brain tubulin and radiolabelled cultured neuronal tubulin show different proportions of the various isotubulins, which shows that the isoelectric focusing patterns are not artefacts of discontinuities in the ampholine *p*H gradient. Previous reports[3] have established that brain tubulin microheterogeniety does not arise from proteolysis during purification. As the carrier brain isotubulin pattern is unaltered in the presence or absence of sympathetic neurone extract (data not shown), degradation during the purification procedure by proteases derived from the neurones is unlikely. Densitometric traces analysed by planimetry revealed 67 ± 4% β-tubulin and 33 ± 4% α-tubulin in the vinblastine precipitates of high-speed supernatants of cultured neurones. When these values are corrected for methionine content (11 for α, 19 for β)[31–33] of the tubulin, we obtain a ratio for α/β close to the 1:1 ratio of α- to β-tubulin found in nonradioactive precipitants from rat brain.

axonal processes. Each cell was labelled with ^{35}S-methionine for 18 h and radioactive tubulin isolated by vinblastine precipitation in the presence of unlabelled brain carrier, then subjected to isoelectric focusing and autoradiography. Figure 2 shows that a single neurone expresses up to seven isotubulins in a pattern very similar to that expressed by mass cultures of 5,000 cells.

We performed several experiments which demonstrated that the isotubulin patterns obtained by one-dimensional isoelectric



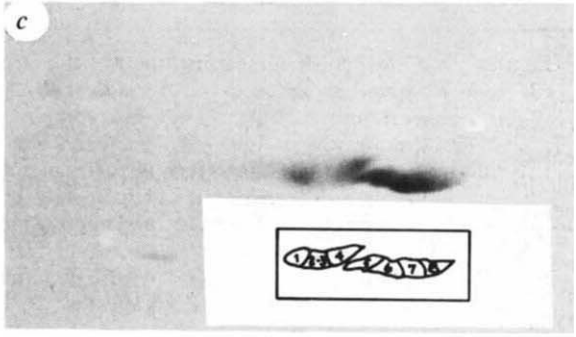


Fig. 3 Two-dimensional polyacrylamide gel electrophoresis (PAGE) of tubulin purified by vinblastine precipitation. The isoelectric focusing gels of mass culture tubulin (Fig. 2) were subjected to electrophoresis in SDS on a gradient of 7–17% polyacrylamide in the second dimension[29]. Gels were stained with Coomassie brilliant blue, dried and autoradiographed as described in Fig. 2 legend. *a*, Coomassi brilliant blue stain of the cold carrier. The contribution of sympathetic neurone tubulin to the stained cold carrier brain tubulin is at the most 5–10% (0.5 μg from the cultures/5–10 μg of cold carrier). *b*, Autoradiogram of the ^{35}S-labelled neuronal tubulin of the same gel shown in *a*; 24 h exposure. *c*, Same as *b*, but a 4-h exposure; enlarged 3×. Inset, spots were numbered by visual inspection. The relative intensities of the spots correspond to the intensities of the isoelectric focusing bands numbered in Fig. 2.

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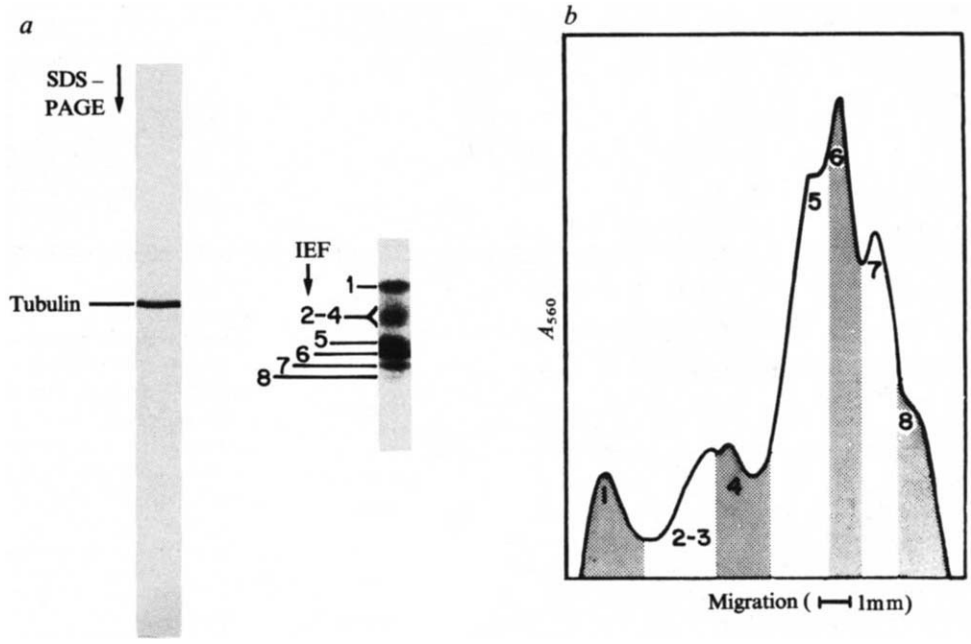

Fig. 4 Tubulin microheterogeneity in sympathetic neurones is independent of the method of purification used. ^{35}S-methionine-labelled tubulin was isolated by DEAE-cellulose chromatography. Mature cells (3–4 weeks in culture) were labelled with ^{35}S-methionine (100 μCi per plate of 2,000–5,000 cells). Cells from five culture plates were collected and mixed with 1.5 ml of brain homogenate prepared in 2 vol of 0.24 M sucrose, 2.5 mM $MgCl_2$ and 50 mM Na-pyrophosphate *p*H 7.0. Cells were sonicated as in Fig. 2 and centrifuged at 30,000 g for 30 min. The resulting supernatant was subjected to DEAE-cellulose chromatography for tubulin purification[3]. *a*, Purified tubulin was subjected to electrophoresis in SDS on a gradient of 7–17% polyacrylamide (SDS–PAGE) as well as to isoelectric focusing (IEF). *b*, The isoelectric focusing gels were densitometrically scanned and shaded areas divided as in Fig. 2*b*.

focusing were not due to contamination by material other than tubulin. Isoelectric focusing and subsequent SDS-polyacrylamide gel electrophoresis on a second dimension (Fig. 3) show that there is considerable tubulin purification by vinblastine precipitation. The gel was scanned in seven consecutive vertical paths within the *p*H range occupied by the various tubulins, and the optical density quantitated by planimetry. Several faint contaminating proteins together comprised 7.4% of the total optical density, and the remaining 92.6% of the label was judged to be contained in tubulin, by the criteria of molecular weight and isoelectric point. No single contaminating protein comprised >1.3% of the total label, and as no single isotubulin band comprised <8.1% of the total label, it is clear that none of the isotubulin bands seen after isoelectric focusing can be attributed to contaminants. Moreover, the contaminating spots were detected only after a 24-h exposure, at which time the tubulin bands were over-exposed. A shorter (4 h) exposure (Fig. 3*c*) reveals multiple tubulin spots corresponding to the isotubulin pattern obtained by isoelectric focusing in one direction (compare with Fig. 2; see also ref. 4); in this exposure most contaminating spots could not be detected (data not shown).

To demonstrate further that tubulin microheterogeneity in sympathetic neurones is independent of the method of purification, tubulin was purified from ^{35}S-methionine labelled mass cultures by DEAE-cellulose chromatography[3]. This technique gave >96% pure tubulin as judged by SDS-polyacrylamide gel electrophoresis (Fig. 4*a*). Isoelectric focusing revealed a similar isotubulin pattern to that found for the vinblastine precipitate (Figs 2 and 4*a*, *b*) and showed the presence of at least seven isotubulins.

These data indicate that a single neurone is capable of expressing virtually the whole range of isotubulins expressed by mass cultures. Although the tubulin microheterogeneity found in sympathetic neurones is comparable in extent with that previously found in brain[3,4,7,14–16], the proportions of the various isotubulins in the cultured neurones are different from those found in the whole brain tubulin carrier (Fig. 2*b*). For example, isotubulins 2 and 3 are greatly enriched in the tubulin of the whole brain. As sympathetic neurones were grown in the absence of non-neuronal cells, this difference could be due to cell or tissue specificity of tubulin forms. Our data cannot determine whether the different proportions of isotubulins in brain and cultured neurones arise from the absence of glia[19,20], the elimination of many neurone types, or differences between newly synthesized tubulin in the cultures and the pre-existing tubulin pool in brain.

The existence of multiple tubulin forms in a single neurone suggests two different hypotheses for specificity in microtubule organization and function: (1) The neurone may have various different forms of microtubules, each containing different isotubulin types. These microtubules may have specialized functions or subcellular distributions. In neurones, the α subunit class is preferentially associated with synaptic membranes[21] and synaptic vesicles[22]. We have preliminary evidence from neurones grown in a three-chamber culture dish (which separates the cell bodies from axons[23]) that there is an enrichment in the soluble β-tubulin class (isotubulins 5–8) in the axoplasm relative to the cytoplasm as analysed by isoelectric focusing. These results are similar to those of Brady *et al.*[24] who found axonal transport of mainly the classically defined β-tubulin by two dimensional gel electrophoresis. In sea urchins, using peptide mapping, Stephens[6] showed that the tubulin subunits of cilia, flagella and cytoplasm have some non-identical peptides, indicating differences in primary structure. (2) Each microtubule in the cell may contain all the isotubulin forms, which may differ by having specific binding sites for regulatory molecules and various subcellular organelles. Microtubule functional specificity may thus result not only from structural differences in the tubulin molecule itself but also from differences in the microtubule associated proteins[25]. Changes in composition and activity of microtubule-associated proteins have been observed during brain development[26] with a time course similar to that of changes in tubulin microheterogeneity[3,11]. It will be of interest to see whether the structural differences between the various isotubulins affect the binding of specific associated proteins and correlate with specific microtubule functions in the single cell.

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E. coli uvrB protein binds to DNA in the presence of *uvrA* protein

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***In vivo* and *in vitro*, the endonucleolytic incision of damaged DNA containing pyrimidine dimers or other bulky lesions requires the action of the *uvrA*, *uvrB* and *uvrC* gene products of *Escherichia coli*[1–3]. To understand better how the products of these three genes mediate incision, all three genes have been cloned[4–10]. Although the gene products of the three genes (designated UVRA, UVRB and UVRC) are all required at the early stages of nucleotide excision repair, it is not known whether they act independently or together as part of a multi-protein complex. As the three proteins are involved in the repair of damaged DNA, it is reasonable to expect that under some circumstances all three proteins should bind to DNA, and, in accord with this expectation, previous experiments have demonstrated that UVRA and UVRC bind tightly to single-stranded DNA[11–13]. We have investigated whether UVRB is also a DNA-binding protein and report here a radiochemical purification of UVRB and experiments using this radiolabelled UVRB to measure its binding to DNA. By itself, UVRB does not bind strongly to DNA, but in the presence of UVRA, tight binding of UVRB to single-stranded DNA is observed, thus demonstrating the occurrence of direct interactions between UVRA and UVRB.**

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We have developed the maxicell procedure for specifically labelling plasmid-encoded proteins, and have used this in combination with transposon inactivation to identify and to purify protein products of cloned genes[7,11,12,14–16]. By comparing plasmid-encoded proteins synthesized in maxicells with those synthesized by derivatives in which the *uvr* genes were inactivated by insertion of a transposon, UVRA, UVRB and UVRC have been identified as polypeptides with molecular weights of 114,000, 84, 000 and 70,000, respectively[11,15–17].

UVRB was labelled in maxicells as described previously[15] and was partially purified so that no other labelled proteins are

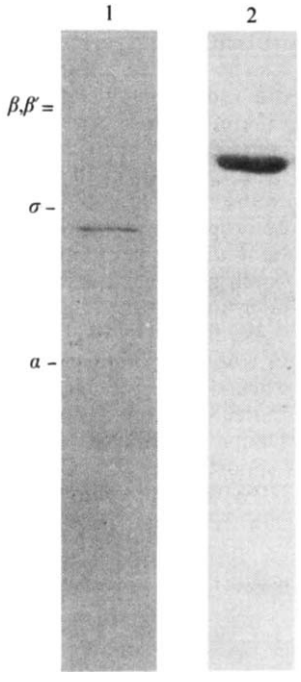

Fig. 1 SDS gels of the preparations of UVRB and UVRA used in DNA binding experiments. Lane 1, autoradiogram of UVRB. ^{35}S-labelled UVRB was prepared from 21 of ^{35}S-labelled (1 μCi ml^{-1} ^{35}S-L-methionine) maxicells of strain CSR603 carrying the *uvrB* plasmid pDR1494. pDR1494 carries the *uvrB* gene and has been described elsewhere[15]. Selective lysis[2,18] was followed by chromatography on DEAE Biogel (Biorad) and Biogel HT (Biorad) to yield a preparation containing 23 μg ml^{-1} total protein and 24,000 c.p.m. ml^{-1} in buffer A (50 mM K-MOPS, 100 mM KCl, 1 mM Na_2EDTA, 10 mM mercaptoethanol and 25% glycerol). Lane 1 shows the autoradiogram of the SDS-gel analysis of 50 μl of this preparation. Lane 2, Coomassie blue-stained gel of the UVRA preparation. UVRA was prepared as described for fraction III in ref. 12 and contained 30 μg protein per ml. Lane 2 shows the Coomassie blue staining pattern of the SDS-gel analysis of 50 μl of this preparation. The subunits of *E. coli* RNA polymerase were run as molecular weight markers (165,000, 155,000, 90,000 and 40,000), and their positions in the gel are indicated in the left margin.

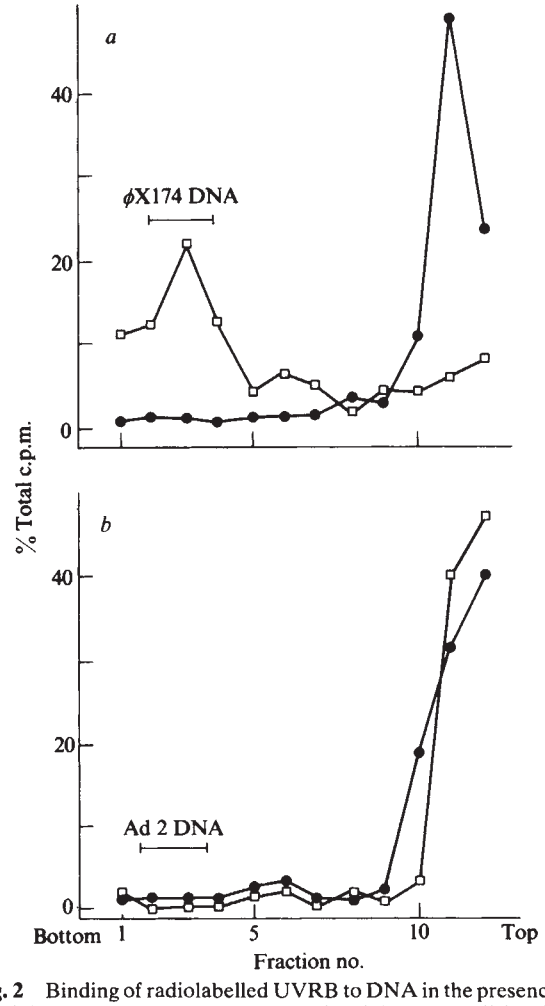

Fig. 2 Binding of radiolabelled UVRB to DNA in the presence of UVRA. 50 μl of the UVRB preparation (Fig. 1, lane 1) is mixed with 0 (●) or 10 μl (□) of the UVRA preparation (Fig. 1, lane 2) with 10 or 0 μl buffer A, 7 μl 1 M $MgSO_4$, 60 μl buffer Y (50 mM K-MOPS pH 7.5, 100 mM KCl, 1 mM Na_2EDTA, 10 mM mercaptoethanol) and 18 μg ΦX174 single-stranded circular DNA (*a*) or adenovirus 2 double-stranded linear DNA (*b*) in 60 μl 10 mM Tris-HCl *p*H 8.0, 1 mM Na_2EDTA. Samples were kept at room temperature for 15 min and then applied to 5–20% linear sucrose gradients (5 ml in buffer Y and 20 mM $MgSO_4$). The gradients are run for 3 h in a Beckman SW50.1 rotor at 45,000 r.p.m. and 25°C after which 0.4 ml fractions are collected and assayed for radioactivity which is plotted above as a function of the gradient fraction. The bar indicates the position of DNA in the gradient.

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detected by autoradiography after SDS-gel electrophoresis (Fig. 1). The radiolabelled UVRB was mixed with either single-stranded or double-stranded DNA and sedimented through a sucrose gradient. The results in Fig. 2 show that there is little or no binding to either form of DNA. We then looked for other factors that might cause binding of UVRB to DNA, being particularly interested in learning whether the addition of purified UVRA would have an effect. UVRA was isolated as described elsewhere[12]. The Coomassie blue-stained SDS gel (Fig. 1, lane 2) shows that UVRA is the only major stainable protein in this preparation. As seen in Fig. 2, when UVRA is present in the mixture, UVRB co-sediments with the single-stranded DNA. As previous studies showed that UVRA had a high affinity for single-stranded DNA, these results indicate that UVRB probably forms a ternary complex with UVRA and single-stranded DNA, because UVRB co-sediments with ΦX174 single-stranded DNA in the presence, but not in the absence, of added UVRA. This provides a mechanism by which UVRB is in the proximity of damaged parts of a DNA molecule even though by itself the protein does not bind to DNA. These results show that UVRA and UVRB interact with each other and strongly suggest that the two proteins act together rather than independently during nucleotide excision repair. The stoichiometry of this reaction and the role of UVRC and other effectors are under investigation.

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Enrichment of satellite DNA on the nuclear matrix of bovine cells

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The skeletal framework of the eukaryotic nucleus is a protein matrix[1,2] which maintains the physical shape of the nucleus. Attached to the matrix is a fraction of DNA resistant to deoxyribonuclease I (DNase I) digestion. Ribosomal DNA sequences also are enriched on nuclear matrices from rat liver[3]. The question thus arises whether other repeated DNAs are preferentially attached to the nuclear matrix. This question could be resolved if the repeated DNA was available in sufficient quantity for biochemical studies and differed in its physical or sequence characteristics from the rest of the nuclear DNA. The satellite DNAs of bovine cells meet these criteria. Here we show that nuclear matrices from bovine cells are in fact enriched in satellite DNA.

Madin–Darby bovine kidney cells[4] were cultured in reinforced Eagle's medium supplemented with 5% fetal calf serum (FCS) and 5% calf serum as previously described[5]. Cells were grown to ~90% confluence on 150-mm Nunclon plates, collected by trypsinization and counted in a haemocytometer. To facilitate nuclear isolation, cells were first incubated for 10 min in a low magnesium buffer: 10 mM Tris-HCl, 0.2 mM $MgCl_2$ *p*H 7.4. This and all subsequent nuclear matrix isolation procedures were carried out at 0 °C. Nonidet P-40 (Calbiochem) was added to a final concentration of 1% and the cells were homogenized with 5–10 strokes of a loose-fitting Dounce glass homogenizer. Nuclei were collected by centrifugation at 750*g* for 8 min and the nuclear pellet resuspended in the low magnesium buffer and again collected by centrifugation. Nuclear matrices were prepared according to the procedure of Pardoll *et al.*[6]. Briefly, purified nuclei were resuspended in the low magnesium buffer followed by slow addition of NaCl to a final concentration of 2 M. To this solution was added DNase I (Worthington) to a final concentration of 200 U ml^{-1} for 20 min. The matrices were collected from this solution by centrifugation at 2,000*g* for 15 min. An example of a nuclear matrix isolated in these conditions is shown in Fig. 1.

DNA was extracted from the purified nuclear matrices by resuspending them in 50 mM Tris-HCl, 10 mM NaCl, 10 mM EDTA *p*H 7.4. T_1 ribonuclease and ribonuclease A (Millipore) were added to final concentrations of 25 U ml^{-1} and 100 μg ml^{-1}, respectively. Matrices were incubated in this solution at 37 °C for 30 min. After RNase treatment, SDS and proteinase K (Boehringer Mannheim) were added to final concentrations of 0.5% and 100 μg ml^{-1}, respectively; incubation was at 37 °C for an additional 30 min. The residual DNA in this solution was extracted twice with phenol and once with diethyl ether. The solution was adjusted to 0.5 M NaCl and the DNA ethanol-precipitated by the addition of 2 vol 95% ethanol. Typically, the residual nuclear matrices contained ~1% of the total DNA when compared with a theoretical value based on the haploid DNA content of the bovine cell (3.3 pg)[7].

The ethanol-precipitated nuclear matrix DNA was resuspended in 10 mM Tris-HCl *p*H 8.0. Solid CsCl was added to an aliquot of this DNA to a final density of 1.715 g ml^{-1}. The DNA was centrifuged at 39,460 r.p.m. for 18 h in a Beckman model E analytical ultracentrifuge. *Micrococcus lysodeikticus* DNA (ρ_{CsCl} = 1.731 g ml^{-1}) was used as a marker. Control DNA was centrifuged in a second model E cell at the same time as nuclear matrix DNA. The isolation of control DNA was identical to that of matrix DNA except that high salt-treated nuclear matrices from control cells were not digested with DNase I. The results of analytical ultracentrifugation of both nuclear matrix and control DNA are shown in Fig. 2.

The bovine satellite DNAs comprise ~15% of the total genome[8]. Of the total matrix DNA, ~60% is satellite DNA and 40% main-band DNA—this represents a fourfold enrichment of satellite DNA on the nuclear matrix. All four major bovine satellite DNAs seem to be protected from DNase I digestion (Fig. 2). To corroborate further the enrichment of satellite DNA on the nuclear matrix, a second aliquot of the isolated matrix DNA was digested with *Eco*RI (see Fig. 3). Fragments characteristic of satellites I (1,400 bp) and IV (970, 1,600 and 2,150 bp) were generated by *Eco*RI digestion (satellites II and III are undigested by this enzyme[9,10]). The relative enrichment of satellites I and IV is also obvious from the reduction in the background DNA due to main-band contamination (Fig. 3, lanes 2, 3). A reported absence of enrichment of satellite sequences on the nuclear matrix[11] may be due to differences in the isolation of matrix-associated DNA compared with methods used here.

A primary role of the nuclear matrix is in DNA replication: the matrix is the site at which DNA replication complexes are fixed[6,12]. DNA is replicated as it moves through the stationary complex[13]. Unlike that of eukaryotes, the bacterial replication complex is attached to the cell membrane[14–16]. The nuclear matrices of SV40-infected 3T3 cells are enriched in SV40

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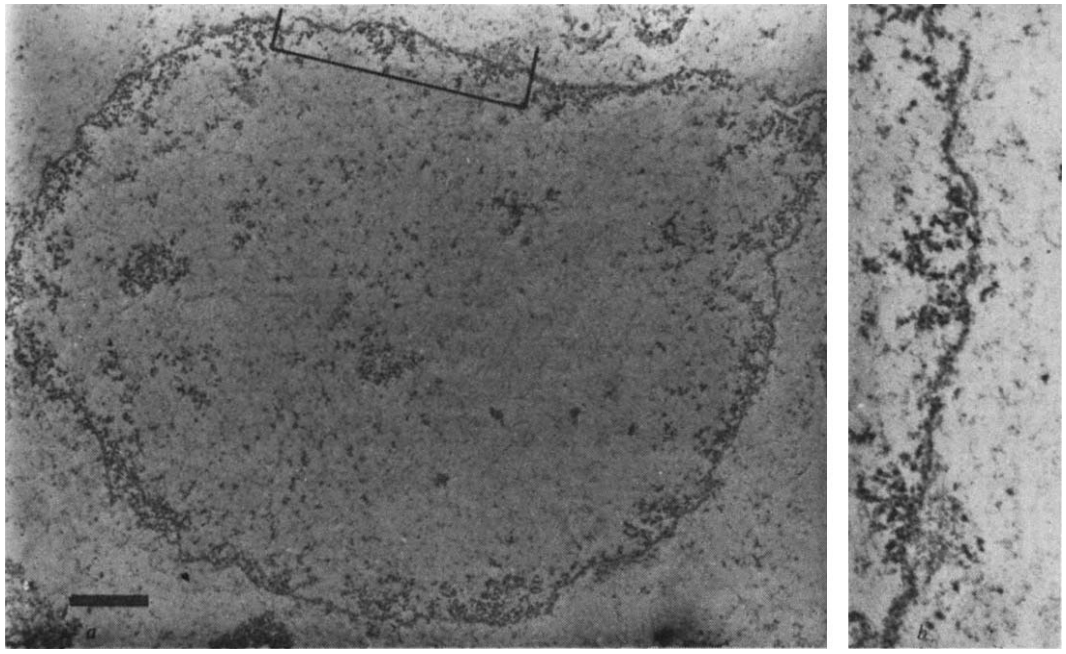

Fig. 1 Electron micrograph of a nuclear matrix from a Madin–Darby bovine kidney cell. Pellets of nuclear matrices were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer *p*H 7.3, post-fixed in 1% OsO_4 and embedded in plastic. Thin sections were stained with uranyl acetate and lead citrate. *a*, The nuclear matrix appears as a thin fibrogranular network. Scale bar, 1 μm. *b*, Enlargement (×1.8) of the peripheral region marked (black lines) in *a*. The periphery appears granular. The absence of a nuclear envelope is due to the use of the detergent Nonidet P-40 in the nuclear isolation procedure.

sequences[17]. This association of viral sequences with the matrix may be due to the recognition and binding of the viral origin of replication by the nuclear matrix. Similarly, the tandemly repeated bovine satellite DNAs[18] may contain periodically spaced origins of replication which are recognized and bound by the nuclear matrix.

I conclude that bovine satellite DNAs are enriched on the nuclear matrices of interphase cells. Whether this enrichment is due to the preferential association of nuclear matrices with origins of replication sequences in satellite DNA or to other satellite DNA sequences is being studied.

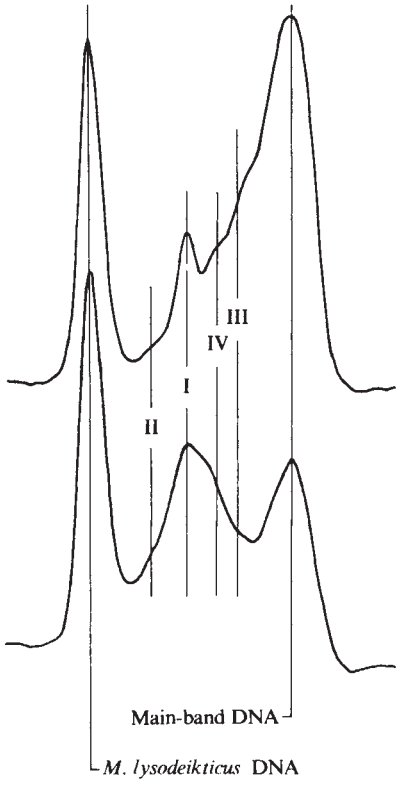

Fig. 2 Analytical ultracentrifugation tracings of control DNA (upper trace) and nuclear matrix DNA (lower trace). The four major density satellites of bovine DNA in CsCl are I = 1.715, II = 1.723, III = 1.705 and IV = 1.710 g ml^{-1}. The percentage of the total DNA attributable to satellite or main-band DNA was determined by cutting out and weighing the respective peaks and regions beneath them.

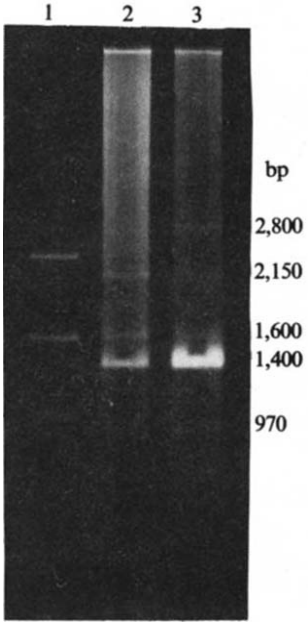

Fig. 3 Gel electrophoresis of *Eco*RI-digested control DNA (lane 2) and nuclear matrix DNA (lane 3). Electrophoresis was in 1% agarose at 70 V for 5 h in 0.04 M Tris-HCl *p*H 8.0, 0.02 M sodium acetate, 2 mM EDTA, 0.018 M NaCl. After electrophoresis the gel was stained with 1 μg ml^{-1} ethidium bromide, and photographed in UV light. The plasmid pBR322, cut with *Hin*cII and *Ava*I, was added to an aliquot of pBR322 cut with *Hin*fI and used as marker. The *Eco*RI fragment of satellite I is 1,400 bp; a dimer (2,800 bp) of this satellite is also present. Satellite IV was cut by *Eco*RI into fragments of 970, 1,600 and 2,150 bp.

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Christmas Books Supplement

What did you read during 1981?

Sixteen contributors discuss the books they particularly enjoyed

Paul Davies

ANY book that opens with the challenge "You may well be wondering if this book is going to be too difficult for you" deserves a few minutes perusal. When, a few lines further on, the author implores "please read at least to page 4" only the most sceptical or faint-hearted reader will lose interest.

After a decade of reading, consulting and reviewing books on the theory of relativity, I found Sam Lilley's treatment of the subject in *Discovering Relativity for Yourself* refreshing and entertaining, as well as a gold-mine of heuristic devices. The informality of style conjures up the image of a draughty Nissen hut in Skegness, which is, no doubt, where much of the material originated — Lilley is an expert on teaching relativity to carpenters, miners and housewives at Adult Education classes. A curious amalgam, this book, of American self-paced inquiry and quaint, Old World, good-for-the-soul instruction.

Glyn Daniel

The Megalithic Art of Western Europe is a book we have been waiting for ever since earlier works on this subject by Coffey and Le Rouzic. It is a corpus and a commentary, and no serious student of prehistoric Europe in the fourth and third millennia BC can afford to be without it — if they *can* afford it at £50.

We have also been waiting for years (indeed it seems to have been ten years in the making) for the first volume of the Cambridge *Agrarian History of England and Wales*. Here it is: Vol.I, Part I *Prehistory*, edited by Stuart Piggott. The editor himself deals with early prehistory, Peter Fowler covers later prehistory — from the Beaker folk to the Romans — and then there is a survey of some 100 pages on the livestock of southern Britain from prehistory to AD 1042 by Dr M.L. Ryder. This book is essential reading for everyone concerned with ancient Britain. □

Ashley Montagu

LYNN Barber's *The Heyday of Natural History 1820–1870* greatly added to the amenities of 1981. It is an utterly delightful romp through a craze for amateur natural history that lasted for 50 years, but which on the whole was curiously unaffected by Darwinism.

Elizabeth L. Eisenstein's *The Printing Press as an Agent of Change* is a unique book and the first of its kind. It is extraordinarily illuminating on the role that printing played in the development of science. I found the book utterly enchanting.

The best book I have read in years on the evolution of humankind is Nancy Makepeace Tanner's *On Becoming Human*. Among its many notable achievements is that it puts the role of women in that development back in its proper place. □

Eric Ashby

AMONG books published over the year, I select two which are straws in the wind of opinion among biologists. One is Robert Boardman's record of the international movement to conserve nature, *International Organization and the Conservation of Nature*. The other is Rupert Sheldrake's *A New Science of Life*, an astonishing challenge to orthodox theories of plant and animal development.

Boardman provides impressive evidence that tens of millions of people (over three million in Britain alone) are enrolled in a campaign to protect nature from hazards caused by mankind; something inconceivable 50 years ago on this scale. Sheldrake's book recalls the writing on holism 50 years ago by Smuts and Whitehead. But he adds a new dimension: he offers to put his hypothesis to the test of experiment. Boardman confirms our attitude of respect for nature. Sheldrake challenges our respect for reductionism. □

Stephen Jay Gould

I WILL cheat a bit and delve back a few months into 1980, since I recommend (in any case) a book for all ages. My choice is, of all things, an index — but no ordinary index. It is the last volume of the monumental *Dictionary of Scientific Biography*, published in 16 parts from 1970 to 1980 with C.C. Gillispie as general editor.

No ordinary, dry accounts of basic facts in the lives of great scientists, these — but intellectual essays (in biographical format) on the social context of science, the nature of creativity and the passion for learning that makes our profession such a joy at its best. Browse and learn. □

Stuart Sutherland

THIRTY years ago it was customary for experimental psychologists to present new work in book form. The publication of Donald Hebb's *The Organization of Behavior* in 1949 and of J.J. Gibson's *Perception of the Visual World* a year later aroused great excitement. Now that psychology has — almost — attained the respectability of a science, those heady days are over. New work appears in journals and most books on the subject merely synthesize existing research.

Of recent psychology books, the two I have most enjoyed are written in non-technical language and can and should be read by workers in all branches of science. In *The Mind's Best Work*, D.N. Perkins summarizes his own research on creativity and by showing that the underlying mental processes are no different from those used in everyday life debunks the mystery of creation that Arthur Koestler and others of his ilk have tried so hard to foster. George Miller's *Language and Speech* presents a lively summary of all aspects of work on language. It has the rare defect of being too short and leaves the reader avid for more. □

Nevill Mott

IN *James Clerk Maxwell: A Biography*, Ivan Tolstoy declares that his subject should be ranked next to Newton and Einstein. He sets out to explain why to the non-scientific reader, who, he says, may well have never heard of Maxwell. Of course, Maxwell's greatest achievement was his equations for the electromagnetic field, and according to the author what was epoch-making in this was the replacement of Newton's action at a distance by the concept of an electromagnetic field.

On reading this book, I enjoyed being reminded of my first acquaintance with Maxwell's equations as an undergraduate, and my feeling that the concept of the displacement current was a supreme example of the power of mathematics to show that what is, must be. I can think of only one comparable example, Dirac's proof that an electron which obeys the laws of relativity and of quantum mechanics must have a fourth degree of freedom. Such things are at the very summit of theoretical physics, and I much enjoyed Professor Tolstoy's attempt to bring this great man's life before us. □

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Lewis Thomas

ON A complicated planet, meaninglessness keeps popping up as explanation: the world makes no sense; we are alone in jumpy randomness, getting nowhere. Amidst this confusion, 1981 provided us with three guide books.

In *Laws of the Game,* Manfred Eigen and Ruthild Winkler glimpse, at a distance, the world at play. Genetic rearrangement, generative grammar and Bach take the same chances, live by the same rules.

Donald Griffin's *The Question of Animal Awareness* is out to show that quick thinking, not all that different from what we think is thinking, abounds. A bee knows what bees are up to. Bats puzzle their way through the dark. Tiny brains can have tiny thoughts.

With high zest, in *Symbiosis in Cell Evolution* Lynn Margulis presents the case for cooperative living as a triumph of evolutionary fitness, an intricate but sure way up life's ladder, the best of games. □

William T. Golden

Would'st thou hear what Man can say
In a little? Reader, stay.*

Statistical Abstract of the United States (101st Annual Edition) presents extensive information on the country's social, political and economic organization. Informative texts introduce the 34 sections (Agriculture, Education, Population, Science, for example); 1,616 tables, 71 charts in 1,059 pages. Something for everyone.

D'ye ken frog from toad, street from road, seer from prophet, amulet from talisman, wasp from hornet; and hundreds more? Then, for fun and edification consult Room's *Dictionary of Distinguishables.*

Leonardo da Vinci: the complex genius, peerless artist, technological visionary, military inventor and opportunist, stirs mind and soul in Martin Kemp's cogently written, generously illustrated analysis. Truly the marvellous works of nature and man. □

*Ben Jonson, *Epitaph on Elizabeth L.H.*

June Goodfield

STEPHEN Jay Gould's *The Mismeasure of Man* achieves several things, one of which is outstanding. By superlative historical scholarship and critical analysis, he demolishes those attempts to limit the lives of other human beings by "measuring" their intelligence whether by the dimensions of their skulls — as in the nineteenth century — or by testing their "supposed" IQs in the twentieth.

His achievement is, first, to demonstrate the strengths and limits of a quantitative measure in science; second, to show that even while science truly *cannot* ever be wholly detached and objective, for prejudices and unconscious attitudes do indeed influence what its practitioners see and interpret, the enterprise nonetheless *does* provide us with a marvellous method for challenging the *status quo*, as well as revealing firm knowledge about the world; third, he reaffirms how much is humanly possible. □

J.Z. Young

RICHARD Leakey's *The Making of Mankind* provides an objective and also beautiful summary of the known evidence about human ancestry. He deals with the facts of the evolution of culture, language and even of civilization and cities, as well as of skulls. He concludes with some wise thoughts about the present, in which he rejects the views of Ardrey, Lorenz and others that human beings are unredeemably aggressive.

This book is a fitting sequel to Solly Zuckerman's classic, *The Social Life of Monkeys and Apes.* When first published, in 1932, it provided many new insights into primate and human reproduction. Now it has been re-issued with a further contribution from the author, who also includes some sensible thoughts about the future. □

A. Hallam

THERE have recently been some exciting developments in palaeontology. For lay readers, especially biologists, who want a clear, non-technical but authoritative account of the study of fossils from an ecological, biogeographical and evolutionary point of view, written in a lively style, 1981 brought Chris Paul's *The Natural History of Fossils.*

For specialists, the textbook by J.R. Dodd and R.J. Stanton, *Paleoecology: Concepts and Applications,* is a sound, comprehensive and well-documented review of recent research — it may well become the standard reference work for some years to come. □

L. Beverly Halstead

I WAS delighted to find in Michael Ruse's new book, *Is Science Sexist?*, a philosopher stoutly defending evolution. Neo-Darwinism is under attack from many directions. The anti-Darwinians seem to be recruiting new forces: including two kamikaze pilots proclaiming *Evolution from Space* directed by a cosmic intelligence from South Wales. At this most critical juncture, we find a new vigorous army, philosophical under Michael Ruse, sweeping across the landscape scattering all before it. I know exactly how Wellington must have felt at Waterloo when Field Marshal G.L. von Blücher and his Prussian troops came into view. The war is not yet over but the final outcome is now assured. Charles Darwin would have been pleased to know you Michael Ruse. □

Isaac Asimov

THE book that pleased and edified me most in 1981 was *Science: Good, Bad and Bogus* by Martin Gardner.

The writing is not strictly that of 1981, for it is a collection of Gardner's essays on various forms of pseudo-science that date back over the past 20 years. Still, aside from the sheer enjoyment Gardner's clear, rational and occasionally sardonic style brings me, 1981 is a year in which his sane voice is enormously important.

Pseudo-science and non-science is on the rise in the United States and the self-named "moral majority" is bringing the force of obscurantist religion into the fight against freedom of thought. Men like Gardner must make their voices heard and in this book he does so most effectively. □

P.B. Medawar

THREE books have struck me greatly during the year. First, C.P. Snow's enthralling *The Physicists*, with an all-star cast. Olympian figures are not necessarily the most interesting or instructive but Snow makes them so because he is big enough himself to see them to scale. Snow himself, shrewd, learned and magnanimous, comes out as well as anyone.

J.M. Tanner's *A History of the Study of Human Growth* is notable as a history of important ideas — seen in a context of intellectual and social history.

Finally, M.F.A. Woodruff's *The Interaction of Cancer and Host: Its Therapeutic Significance* is a *tour de force* with the breadth and depth of a multi-author volume, yet the work of a single first-rate mind. □

Eugene Garfield

I MET V.V. Nalimov, the well-known Soviet statistician and cybernetician, at the first Moscow Book Fair. We were brought together by our mutual, long-standing interest in scientometrics. After I read his manuscripts, I recommended that we should publish two of his books.

In the Labyrinths of Language: A Mathematician's Journey investigates the languages of biology, art, mathematics, statistics and philosophy. Nalimov proposes a taxonomy of languages, which describes the spectrum-continuum of language, from the "hardest" (mathematics) to the "softest" (art, Hindu metaphysics).

Nalimov ranges even more widely in *Faces of Science.* In these essays, each reflecting a different aspect of science, he demonstrates that science is a large, self-organized information system. How could I, as a linguistics-trained, essay-writing, information scientist, resist these fascinating ideas? □

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Details of publishers and prices

Agrarian History of England and Wales. Vol. I, Part I Prehistory. (Cambridge University Press.) £27.50, $64.50.

Dictionary of Distinguishables. (Routledge & Kegan Paul.) $12.95, £5.95.

Dictionary of Scientific Biography. (Charles Scribner's Sons.) 16 volumes, $595. Single volume concise edition $100.

Discovering Relativity for Yourself. (Cambridge University Press.) Hbk £17.50, $49.50; pbk £7.95, $19.95.

Evolution from Space. (Dent.) £7.95.

Faces of Science. (ISI Press.) $22.50 US, $25.50 elsewhere.

The Heyday of Natural History. (Cape/Doubleday.) £9.50, $17.95.

A History of the Study of Human Growth. (Cambridge University Press.) £30, $69.

In the Labyrinths of Language: A Mathematician's Journey. (ISI Press.) $22.50 US, $25.50 elsewhere.

The Interaction of Cancer and Host: Its Therapeutic Significance. (Grune & Stratton/Academic.) $46.50, £26.20.

International Organization and the Conservation of Nature. (Macmillan Press.) £15.

Is Science Sexist? (Reidel.) Hbk Dfl.80, $42; pbk Dfl.32.50, $14.95.

James Clerk Maxwell: A Biography. (Canongate.) £9.95.

Language and Speech. (W.H. Freeman.) Hbk £8.20; pbk £3.50.

Laws of the Game. (Knopf.) $19.95.

Leonardo da Vinci: The Marvellous Works of Nature and Man. (Harvard University Press/Dent.) $30, £14.95.

The Making of Mankind. (Michael Joseph/Dutton.) £9.95, $24.95.

The Megalithic Art of Western Europe. (Clarendon/Oxford University Press.) £50, $85.

The Mind's Best Work. (Harvard University Press.) $18.50, £12.95.

The Mismeasure of Man. (W.W. Norton.) $14.95.

The Natural History of Fossils. (Weidenfeld & Nicolson/Holmes and Meier.) Hbk £15, $37.50; pbk £6.95.

A New Science of Life. (Blond & Briggs/Tarcher.) £12.50, $11.95.

On Becoming Human. (Cambridge University Press.) Hbk £20, $29.95; pbk £6.95, $10.95.

Paleoecology: Concepts and Applications. (Wiley.) £29.55, $53.15.

The Physicists. (Macmillan Press/Little, Brown.) £8.95, $15.95.

The Printing Press as an Agent of Change. (Cambridge University Press.) Pbk £12.50, $16.95.

The Question of Animal Awareness. (Rockefeller University Press.) $13.95.

Science: Good, Bad and Bogus. (Prometheus.) $18.95.

The Social Life of Monkeys and Apes, 2nd Edn. (Routledge & Kegan Paul.) £17.50, $55.

Statistical Abstract of the United States (101st Annual Edition). (US Government Printing Office.) Hbk $14, pbk $11.

Symbiosis in Cell Evolution. (W.H. Freeman.) Hbk $24.50, £16.40; pbk $14.95, £9.20.

Archaeoastronomical anomalies

Clive Ruggles

The First Stargazers: An Introduction to the Origins of Astronomy. By James Cornell. Pp.262. ISBN US 0-684-16799-9; ISBN UK 0-485-30004-4. (Charles Scribner's Sons/Athlone: 1981.) £7.95, $15.95.

ARCHAEOASTRONOMY is a new and potentially exciting discipline. Its precise aims — broadly, investigation of the astronomical practices of ancient societies through various aspects of their material legacy — and limitations are not (yet) well defined; it includes on the one hand studies of Mesoamerican structures, where site evidence can be considered alongside written evidence from codices and ethnohistoric evidence, and on the other studies of British megalithic sites, where conclusions depend almost entirely upon the statistics of alignments and horizon indications. Almost all workers in the field are part-time, retired or amateur, and their main interests cover a variety of academic disciplines. The scientific value of much archaeoastronomical evidence has still to be properly determined; methodological improvements are only now generally being made. Thus the status of much potentially interesting field work has yet to be reassessed and confirmed.

All of this presents the popular writer on archaeoastronomy with a number of problems. First, he has to cope with a confusing diversity of material, geographically and in terms of approach and quality. Second, he has to give some account of the difference between testable hypotheses and valueless speculation, and distinguish between different theories on these grounds. Finally, he has to combat the many popular misunderstandings, and be careful not to be drawn into endorsing purely speculative or now-refuted ideas on the grounds that they provide exciting material and help to sell books.

On the first count James Cornell has coped very well indeed; material is organized into chapters on a geographical basis, and general points are made whilst discussing particular sites or theories. However on the second and third counts the book is a mixed bag, indeed: on occasions the author has done an excellent job, but on others he has failed dismally, and, worse, will badly mislead.

There are many instances in the book where valid theory is well separated from mere speculation, and the point is often made that deliberate astronomical orientations need to be distinguished from mere chance occurrences. In the opening descriptions of equinoctial sunset over a pyramid at Chichén-Itzá in Mexico, Cornell follows a description of a shadow phenomenon by asking "is this merely coincidence?" and explaining why he thinks it is not. Later he uses the example of a rock carving at Toro Muerto in Peru to illustrate a point about subjectivity and different possible interpretations. In the chapter on Egyptian temples, a reasonable approach (by Gerald Hawkins) is well distinguished from the speculations of various "pyramidologists". A good account is given of both sides of an argument about the astronomical interpretation of rock art. The "Sirius mystery" of the Dogon tribe in Africa, and its probable explanation in terms of earlier contact with someone from the outside world, is entertainingly recounted. And so on.

The author is not, however, always so clear about why he is ready to endorse some theories and dismiss others, and does not always identify the more speculative theories as such. Thus we hear that Alexander Marshack's ideas on lunar notation amongst bone scratches from the Cro-Magnon period are "steadily winning support throughout the scientific community". There is scarce mention of a more objective approach, which might ask how many other interpretations could equally well have been placed upon the bone marks. Instead, we are told how a "background as a journalist, screenwriter and popularizer of science does not help win support in the scientific community". The author might reflect that in some cases this might be due to an inadequate apprec-

British Tourist Authority

Stonehenge sees another dawn — and continuing debate over how the stones and their alignments should be interpreted.

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iation of the rigours of the scientific method.

Mr Cornell is at his most dogmatic in the chapter on Stonehenge, which is disappointing and, indeed, infuriating. Here we see regurgitated the Stonehenge debate of the 1960s, with Gerald Hawkins hailed as "having refuted nearly a century of archaeological dogma" and "having begun a revolution that would lead to a complete rethinking of the intellectual development of humankind", while archaeologists such as Richard Atkinson are denigrated for daring to criticize, and are said to be motivated by their "traditional view of these people as backward barbarians". In the same chapter Lockyer's earlier theories are discussed and justly criticized, but when it comes to Hawkins there is suddenly a flagrant disregard for any objective discussion. Instead, as one encounters more and more references throughout the book to Hawkins's "pioneering research at Stonehenge", how he has been praised as the "father of archaeoastronomy", and how he "almost single-handedly changed public and scientific conceptions of archaeoastronomy", one begins to wonder if this isn't really a Gerald Hawkins tribute volume.

It is certainly true that Hawkins's work on Stonehenge led to a general public interest in archaeoastronomy and it is undeniable that Hawkins has done much work of value in this field, but the work on Stonehenge itself was soon criticized on justifiable grounds. It is perhaps worth (tiresome though it is to have to do so, for it has been done several times) briefly putting the record straight here. The astronomical interpretation of Stonehenge I rests on the finding that 24 out of 50 possible alignments between pairs of features at Stonehenge I were of astronomical significance. The chances of this happening fortuitously were calculated by Hawkins to be less than one in a million, as quoted by Cornell. In fact, there were 24 lines out of a total possible of over 100, since alignments both ways along most pairs were allowed as possibly astronomical; Hawkins calculated the probability of exactly 24 astronomical "hits" rather than 24 *or more*, and different tolerances were allowed on different lines. When these errors are taken into account, no evidence whatsoever remains for preferential astronomical orientation. This is quite apart from any archaeological and other arguments about the choice of features for inclusion in the analysis. These errors are simple ones — certainly not a case for "mathematicians still arguing" as stated by Cornell — and, although they were pointed out by Atkinson only a year after the publication of Hawkins's *Stonehenge Decoded* (Doubleday, 1965), even now they are still present in current reprints of the book. This fact does not imply any great commitment to objective research, and it is tragic that Cornell's book, if widely read, will only revitalize public misconceptions on this point. Cornell rightly points out that Hawkins's conclusions "never seemed to bother a technically attuned public": with the exception, one may surmise, of those with a questioning mind and some high-school mathematics.

In the chapter on Stonehenge and British archaeoastronomy in general, the author dismisses the archaeoastronomical work of Professor Alexander Thom in less than three pages, and mentions later that Thom's work scores a very low "reliability value" according to criteria devised by Hawkins (under which, not surprisingly, Hawkins's own work scores a high value). This sort of denigration is insidious. Thom has investigated a large number of sites, presented his results in a manner so that they can be readily re-examined and criticized and has been prepared to modify his opinions in the light of criticism and new evidence. (I am amongst those attempting critical assessments of his work.) In this manner, hypotheses are presented, criticized and modified, a procedure in accord with the scientific method but perhaps less susceptible to large-scale popular interest.

There are a number of minor errors in the book which there is no room to mention in detail, particularly amongst the astronomical background material cunningly concealed amidst discussion of Toro Muerto and Marshack's work (that it *is* cunningly concealed is not a criticism).

But the overriding criticism of this book must be its one-sided and misleading treatment of British archaeoastronomy, in particular the almost fanatical praise of Hawkins and detriment of his critics. The complete break with objectivity in this respect must invalidate the value of the rest of the book, at least for non-specialist readers (and this is the intended audience) who cannot judge for themselves. Indeed, it will quite possibly have a worse effect, for in raking up past confusions and prejudices, amongst an objective discussion of other fields of archaeoastronomy, together with its several references to the prejudice of archaeologists against new ideas, it threatens to direct popular support towards sensationalism and away from level-headed scientific research. □

Clive Ruggles is University of Wales Research Fellow in the Department of Archaeology at University College, Cardiff.

Exploring Britain's prehistoric past

Ruth D. Whitehouse

The Penguin Guide to Prehistoric England and Wales. By James Dyer. Pp.384. ISBN 0-7139-1149-6. (Allen Lane: 1981.) £9.50.

POPULAR interest in archaeology in the British Isles has never been higher, an interest which has brought increasing numbers of visitors to the visible remains of our past, our ancient monuments. James Dyer's book is a concise guide to almost a thousand of the most interesting sites of the prehistoric period in England and Wales. It is not the only guide of its kind: competitors include Nicholas Thomas's *Guide to Prehistoric England* (Batsford, 2nd Edn 1973), Richard Wainwright's *A Guide to the Prehistoric Remains in Britain: South and East* (Constable, 1978) and James Dyer's own *Southern England: An Archaeological Guide* (Faber, 1973).

The advantage of the new Penguin guide over these other works is its wider geographical coverage and the larger number of sites included. It begins with a section of five maps and a short introduction to British prehistory. The main part of the book consists of a gazetteer of sites, listed alphabetically by county. The gazetteer itself is excellent: the accounts of sites are concise and accurate, with Ordnance Survey grid references included; many of the site descriptions include one or more bibliographical references and some are illustrated with photographs or line drawings. The visitor is therefore provided both with clear descriptions and an introduction to further reading.

Less satisfactory is the introduction. Archaeology today is developing fast and is characterized by controversy and debate on almost every topic. Dyer ignores this and presents a consensus view of British prehistory, which may accurately reflect majority opinion, but which neglects the new approaches and hypotheses that have transformed the subject in recent years. To take a single example, the Beaker pottery and associated artefacts of the third millennium BC are explained in terms of people migrating into and across England. This is indeed the traditional interpretation, but there is an alternative view that they represent a "status kit" of prestigious objects which were acquired by influential and wealthy people in different societies and were distributed not by migration, but by trade or exchange. While a guide book is not perhaps the proper forum for the airing of controversy, the onus surely remains to provide an up-to-date level of interpretation. Nonetheless, this is a book that everyone interested in Britain's prehistoric past will wish to own. □

Ruth D. Whitehouse is a Lecturer in Prehistoric Archaeology at the University of Lancaster.

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Odds and ends about the dead and buried

Stuart Piggott

Rites of the Gods. By Aubrey Burl. Pp. 258. ISBN 0-460-04313-7. (Dent: 1981.) £12.

WITH its meretricious title and dust-jacket, is Mr Burl's book going to lead us to take the "misdirected step" that "leads one towards apparitions, White Goddesses, psychic archaeology and the Never-Never land of the ley-liners"? Of course not: the deservedly caustic phrases are those of the author. But his book is not quite what it might seem to be, for it is not a discussion of ancient non-Christian cults, not even of those inferred for prehistoric Europe, but is limited in space to the British Isles and in time to a period *c.* 4500–1000 BC, with sketchy chapters for the phases before and after. Within these limitations Mr Burl presents to the non-archaeological reader some of the inferences which can be made as to ritual practice and performance from the material culture of burial and ceremonial monuments, a field to which he has made important contributions.

In the six chapters which form the bulk of the book the treatment is not thematic but topographical, with what seems a capriciously selected series of field monuments, some surviving, some destroyed or invisible from the ground. This does not make for easy or even very interesting reading, and obscures the real topics which could have been explored, such as the variations perceptible in the rites of collective burial — successive, re-deposited, partial or complete — or a fuller treatment of the claims for astronomical expertise in British prehistory, on which the author has made pertinent comments elsewhere that deserve re-statement. Unfortunately, too, there are many errors of detail in the site descriptions, and the reports of early antiquarian diggings are sometimes given more credence in detail than they deserve. Modern "folklore" is also dealt with uncritically and without regard to the absorption by popular tradition, from the Renaissance onwards, of literate antiquarian speculations. Even less sure is the handling of anthropological evidence, where a sprinkling of words such as "shaman", "totem", "mana" or "sympathetic magic" hardly inspires confidence, and Ucko's classic paper of 1969 (*World Archaeology* **I.2**, 262–280) on precisely the problems posed by the interpretation of prehistoric funerary practice is not mentioned, though it should be required reading for all concerned. The limitations of inference from "grave archaeology" are nowhere set out.

Surprisingly, when in the later first millennium BC something, however tenuous, becomes explicit in Celtic religion, the book tails away. Here it is strange, in view of the prominence rightly given to henge monuments and allied structures earlier in the book, that nothing is said of the evidence discussed in several recent papers for an insular survival of this pre-Celtic sanctuary tradition in such sites as Dun Ailinne or Emain Macha (and Tara), with probable British counterparts; the circular timber Iron Age temple beneath the Roman structure on Hayling Island is among other new and important cult sites of the period.

All in all, *Rites of the Gods* cannot be called a success, and does little more than present the reader with a scrap-book of miscellaneous data, too often interpreted in terms of modern modes of thinking. I had expected, and the general public deserves, something much better from Mr Burl. □

Stuart Piggott retired as Abercromby Professor of Archaeology at the University of Edinburgh in 1977. His books include Ancient Europe *(Edinburgh University Press, 1965) and* The Druids *(Thames & Hudson/Pelican, 1974).*

Jupiter and the Moon displayed

David W. Hughes

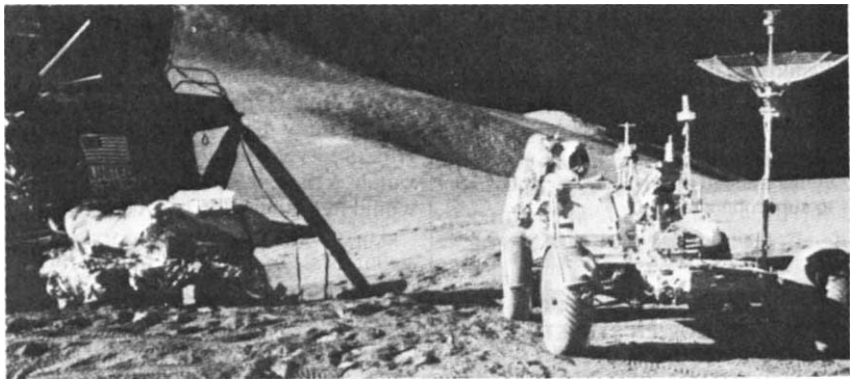
I FIND map books fascinating and spend many happy hours investigating a specific country and tracing the lines of equal height, geological structure, nationality, temperature, rainfall, population, magnetic inclination and so on. The fascination seems to be a function of the amount of information displayed, so turning to the planets the first question is, "Do we know enough to fill a good atlas?". Looking at the two atlases under review one also must ask if all the relevant information has been included.

The first two volumes in The Mitchell Beazley Library of Astronomical Atlases for Amateur and Professional Observers deal with Jupiter and the Moon. *Jupiter* has been written by Garry Hunt and Patrick Moore, who divide their attention equally between the planet and its satellites. There is no doubt about there being enough knowledge to fill an atlas here. The images from Voyager 1 and 2 and Pioneer 10 and 11, apart from revealing three new satellites and a ring around the planet, have provided a host of superb pictures. This atlas reproduces many of these in colour. It not only provides maps of Jupiter and its satellites, but it also reviews our knowledge of such topics as The Great Red Spot, the interior of Jupiter, the magnetosphere, the effect of Io on Jovian radio emission, cloud morphology and colouring, volcanism on Io, surface fracturing on Europa, and cratering on Callisto and Ganymede.

The book is well written, physical and astronomical terms being clearly and correctly defined.

After the excitement of the Pioneer and Voyager fly-bys we are now scientifically becalmed and must return to "old-fashioned" visual and photographic observations from Earth. No Earth-based telescope can show anything like the amount of detail provided by space imaging, but Jupiter can be kept under observation for a large fraction of each year. It is thus well worth mentioning that the bible of Jupiter watchers — Bertrand M. Peek's *The Planet Jupiter, The Observer's Handbook* — has been re-issued in a revised edition (Faber and Faber; £10, $29.95). Needless to say, with 170 pages devoted to observations of the belts and zones of Jupiter and their temporal variation, this book is not for the faint hearted.

The second atlas, *The Moon* by Patrick Moore, reflects, to quote the author, "the endless enjoyment to be gained simply from looking at the Moon". Despite being visited by astronauts and a multitude of unmanned probes, the Moon has lost nothing of its romance. Again, the atlas contains about 16 pages of colour plates and a large collection of stereographic, orthographic and Mercator maps of lunar regions.



The mobile man in the Moon — James B. Irwin and the Lunar Roving Vehicle. The picture is taken from Joseph Jackson and John Baumert's *Pictorial Guide to the Planets*, 3rd Edn, just published by Harper & Row, price £9.50.

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Moore provides a basic introduction to crater morphology, lunar chronology, mascons, transient lunar phenomena, regolith formation and the recent space exploration programmes. I would have liked to have seen one or two coloured geological maps (a typical example could have been the US Geological Survey map of the Copernicus quadrangle) and maybe a detailed contour map would have been fun (NASA's lunar topographic orthophotomap of the Apollo 15 Hadley region would do). Additional detail about surface temperature variation, transient atmospheres and the present meteorite influx rate would also not have been amiss.

We all have our favourite crater and I was surprised that mine, Giordano Bruno (which *might* have been formed as recently as July 18, 1178), doesn't even rate a mention. However Moore has produced an ideal book for the amateur observer, and anyone who ever fancies turning a telescope or a pair of binoculars onto the Moon will find this atlas an excellent starting point and guide.

Despite the grandiose title given to the series by Mitchell Beazley I think the two atlases, especially *The Moon*, fall well into the amateur division of the astronomy book league. I must say, though, that at £6.95 each (in the US $14.95, Rand McNally) their value for money will be hard to beat. □

David W. Hughes is a Lecturer in Physics and Astronomy at The University of Sheffield.

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Guidance for the observant amateur

Patrick Moore

A Complete Manual of Amateur Astronomy. By P. Clay Sherrod. Pp. 319. ISBN hbk 0-13-162115-7; ISBN pbk 0-13-162107-6. (Prentice-Hall: 1981.) Hbk $24.95, £18.70; pbk $10.95, £8.20. *The Practical Astronomer.* By Colin A. Ronan. Pp.153. ISBN UK 0-333-31245-7; ISBN US 0-02-604500-1. (Macmillan, London/Macmillan, New York: 1981.) £8.95, $20.

ALTHOUGH no book can be really regarded as complete, Clay Sherrod's *Manual* is certainly exhaustive, and it contains much up-to-date information which cannot be found elsewhere in convenient form. The author is a well-known amateur astronomer, and his experience will be found to be very useful to others.

Topics covered range from telescope selection and maintenance to naked-eye work (meteor observation, for example); studies of the Moon and planets; photoelectric photometry, and all kinds of astronomical photography. The sections dealing with equipment are of special value. Many amateurs tend to believe that setting up a photoelectric photometer is too much of an undertaking; reading this book may cause a change of outlook. The author stresses, rightly so, that the modern amateur has to be much more specialized than his predecessor of half a century ago.

There are, unfortunately, some blemishes, and the weakest parts of the book are those dealing with the Moon and planets. In the lunar section, no mention is made of transient lunar phenomena, even though it is here that the amateur can carry out his most valuable work. In some ways the information given is out of date; for instance, it is no longer believed that lunar rills are "dried beds of water flow" — this idea was abandoned many years ago, and analyses of the lunar samples brought home by the Apollo astronauts and the Russian Luna probes have shown that there are no hydrated materials on the Moon. The map of Mars given on p.141 is, frankly, of no use, and the list of Martian features includes many canals and other markings which do not exist at all — so that the amateur will be hard pressed to locate them! The most surprising statement is that in Saturn's system, "the satellites Titan, Mimas and Hyperion are those that you should study most during transit events". Any observer using a modest telescope to watch a transit of Mimas or Hyperion would indeed have to have keen vision.

There are comparable mistakes in the stellar lists. On p.204, it is said that Antares varies from magnitude 0.9 to 1.8 — in fact its fluctuations are too slight to be detected without using a photometer or some such device; Alpha Cassiopeiae never rises to magnitude 1.4, and even its variability is questionable (Kukarkin's catalogue omits it); presumably *n* Aquilae should be η Aquilae, R Geminorum should be U Geminorum, and so on. These errors are trivial in themselves, but in a book aimed at providing a guide for amateurs they can be highly misleading.

All in all, it seems that the proof-reading has been inadequate, and that the author is not fully conversant with some of the latest research. But the merits of the book far outweigh the defects, and there is nothing which cannot be put right in the next edition. When this has been done, the book may well become a standard work.

Colin Ronan's book, *The Practical Astronomer,* is much more elementary, and aimed at the younger reader. As well as providing a general introduction to astronomy, it contains many practical demonstrations and experiments; the text is clear and riveting, and the illustrations excellent. A book to be strongly recommended. □

Patrick Moore is an author and astronomer. His recent books include The Moon, Jupiter *(with Garry Hunt) and* The Atlas of the Universe, *all published by Mitchell Beazley.*

Cosmic methadone

William H. Press

The Edge of Infinity: Naked Singularities and the Destruction of Spacetime. By Paul Davies. Pp.194. ISBN UK 0-460-04490-7; ISBN US 0-671-44063-2. (Dent/Simon & Schuster: 1981.) £7.95, $14.50.

"BEYOND black holes to the end of the universe", trumpets the cover blurb. The author, with more modest affect, relates a conversation among colleagues bemused at the plethora of black-hole books: " 'Whatever next?' someone asked. 'The naked singularity!' came a prompt reply. So I have written, with some amusement, a book about naked singularities".

A naked singularity, in modern relativity theory, is a region of spacetime where gravitational forces become infinite, crushing matter to infinite density, but from which light signals can escape, rendering the singularity visible. The singularity inside a black hole is not "naked" — the choice of this word is usually attributed to Roger Penrose — but is, rather, modestly clothed behind an opaque "event horizon" (the surface of the black hole) through which outward light signals cannot propagate. The big-bang singularity from which our Universe expanded is, however, naked: the 3K cosmic microwave background is a signal from that singularity (or, at least, from as close to that event as the opacity of dense, ionized matter will allow us to look).

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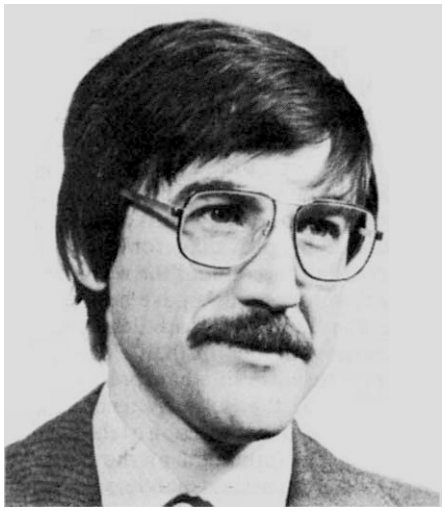
In claiming to have given us a book "beyond black holes", the author is gently pulling our legs. Really, the book *is* another black-hole book — quite a fine specimen of the genre — and it is also about all those other popular buzzwords which have leaped from relativity theory into the public domain: warped spacetime, gravitational collapse, the big bang, Schwarzschild radius, Hawking radiation and so on.

The market for these books seems as insatiable as that for Rubik's cubes. It is out of this crowded pack that Paul Davies has emerged as a prolific and, I think, major figure in popular science writing. This book is his sixth or so in less than five years. They keep getting better and better. Davies writes with a clear, sure, sane voice, at once understandable and astonishingly close to the Nirvana of total technical accuracy.

There are other good, popular black-hole books of course. One, Iain Nicolson's *Gravity, Black Holes, and the Universe,* was praised by Paul Davies recently in *Nature* (**290**, 656) in phrases even more applicable to Davies's own book: ". . .maintains a reliable scientific perspective and a careful treatment, yet covers most of the 'fun' topics in detail". What sets Davies's book apart is not just that it is good, but that it is determinably good, written by an author with a now-established corpus of good science writing behind him and (one hopes) with more still to come. Librarians can confidently put him on their automatic-purchase lists.

Who are Davies's readers? One understands, to be sure, that there are two entirely distinct audiences for popular science, each blissfully ignorant of the other's existence. Group A, beloved by "serious" science popularizers, is imagined to consist entirely of educated country gentlemen and intelligent high-school students. Group B, the targets of science writers who want to make some money, are the gee-whizz junkies, uncritically addicted to the wildest and most irresponsible sorts of speculation. Astonishingly, I think that Paul Davies, in this book, will appeal to both groups. He is onto something that is either a great scam, or a great advance, in science popularization: the Group B addict is lured by a flashy pitch, promised the excitement that he so badly needs. What he gets instead is a sane and accurate exposition, a detoxification instead of a "high". The Group A reader, meanwhile, not only finds the pitch amusing, but also the actual content entirely satisfactory. □



Paul Davies — "a clear, sure, sane voice" for both country gentlemen and the gee-whizz junkies.

William H. Press is Professor of Astronomy and of Physics at Harvard University.

A new dimension to evolutionary theory?

D.A.J. Tyrrell

Evolution from Space. By Fred Hoyle and Chandra Wickramasinghe. Pp.176. ISBN 0-460-04535-0. (Dent: 1981.) £7.95.

THIS is in many ways an attractive book. The cover suggests the sky at night, but it is actually a photograph of "cell bodies in mollusc" culled from *Nature* — a clever visual linking of the ideas in the book. And the book is clever too; it ranges with relaxed ease over some biological oddities, such as the fact that *Drosophila* responds to UV light of wavelengths too short to be found on Earth, that *Vicia faba*, a member of the pea family, makes haemoglobin and that some bacteria are incredibly resistant to radiation. In style it seems intended for the general reader, and expounds a grand idea rather than presenting a closely argued case.

The idea is that biological evolution is widely regarded as proved and a complete explanation for the living world as we see it — yet there are awkward facts such as those I have just mentioned which to Hoyle and Wickramasinghe show that genes borne on particles in space have been incorporated into organisms on Earth. They also think this explains the sudden appearance of new species and the existence of gaps in the fossil record. They used essentially the same idea to "explain" in their earlier writings what they regarded as inexplicable features of infectious diseases; then the explanation was germs from space.

The authors make their own low estimates of the frequency of certain mutations and of the improbability that the amino acid sequence of any biologically significant protein could arise "by chance" in a primaeval soup. They then resolve the dilemma by mentioning Arrhenius' theory of "panspermia" and reformulating it by saying that "biomaterial" is parked on particles in space protected by low temperature and low gas concentration, and defended by graphite from radiation. They argue that the material on particles in a certain size range would remain viable after impinging on Earth, as described in their earlier books. In Chapter 7 this is likened to sprinkling new "computer programs" into computers in the planetary system and beyond — that is, new genes into species living throughout the Universe. They point out that studies which apparently showed unexpectedly large numbers of bacteria in the upper air were discounted by NASA as due to contamination and were not followed up.

I think I understand the dilemma which made Hoyle and Wickramasinghe write the book. The intellectual difficulties of conceiving how life began and developed are stupendous, and the facile suggestions they sometimes include make one doubt the plausibility of some of the proposed schemes — in my own field I cannot see viruses as the "original" life since all those we know need energy and translation systems taken from whole cells in order to reproduce.

So, we agree that is difficult to explain how the first life forms were put together and why the fossil record suggests that either there were no links between certain species or evolution went very fast on a geological time scale. But this book merely moves these problems without solving them — for how were the genes in space created in the first place? And how, once landed on the Earth, were they incorporated into the genome of species already here? Genetic engineers can create environments in which genes can be implanted from without but the likelihood of this happening with extraterrestrial material in a natural environment is to me, intuitively, much less than that of evolution from time to time going so fast in so few animals that no fossil record has been found.

The authors are apparently sympathetic to the idea of a creative God, though most of the references are to one or more "intelligences", designated as ?, ?? and so on. I get the impression, for many important parts of their structure are not defined, that although they refer to the old theory of whole planets being disrupted and sending their germ plasm into orbit, the authors lean towards a concept of biomaterial being formed, perhaps *de novo*, in many parts of the Universe — a sort of biological continuous creation. But that again is moving the problem about in time, not solving it. I am afraid their hypothesis reminds me of the theological view labelled as "The God of the Gaps"; in fact they admit that their views are a form of the theory of special creation. Such theories bring in a God when the intellectual going gets tough and manage without between

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times. It seems to me that it is very likely that the Universe *is* the creation of a God, but in that case I take the view that the whole grand structure, the bits I think I understand, as well as the bits I certainly don't, are and always have been manifestations of the same intelligence and creating and sustaining power.

On the same basis, although a Creator obviously *could* at any time or place suspend the "laws of nature" as we have observed them, we can use the method and approach of science only if we assume that these laws operate consistently in time and space. Here the argument echoes that of the "creationists" and "evolutionists" in the USA. It might happen that a scientific examination *forced* the conclusion that, apart from the existence of the Universe in time and space, some other specific creation events or insertions of genes from space could be demonstrated to have taken place. Hoyle and Wickramasinghe have not convinced me — though my mind is still open. □

D.A.J. Tyrrell is Deputy Director of the MRC Clinical Research Centre, Harrow, Middlesex.

To be an astronomer

Stephen P. Maran

In Quest of Telescopes. By Martin Cohen. Pp.131. ISBN 0-9333-4625-5. (Sky Publishing, Cambridge, Mass: 1981.) $11.95.

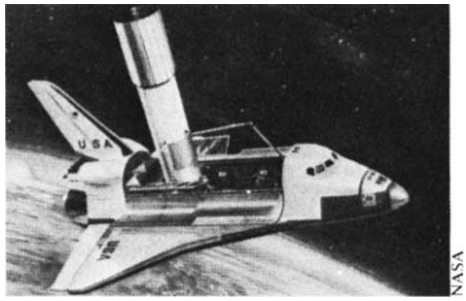
MARTIN Cohen, an expert on T Tauri stars and cometary nebulae, and an English astronomer in the grand tradition of observation and discovery, has written his "astronomical autobiography". Unashamedly still an amateur at heart, Cohen is one of the diminishing breed of professional astronomers who actually enjoy the view through the eyepiece while they make the arcane measurements of the trade.

The book describes a life-long love of telescopes and star-gazing by way of vignettes drawn mostly from time spent at the major observatories of the American West. A photograph of the awed author, dwarfed by the 200-inch Hale reflector, is symbolic representation of the principal theme, a paean to the beauty both of the stars and of the great instruments with which we study them. Helicopter rides in the snow-bound White Mountains aside, his travels to pursue observational research at remote sites in North and South America are typical of those of many astronomers of the present generation who, as Cohen says, "have become jet-set commuters".

Although I began ten years before Cohen and on the opposite shore of the Atlantic, I see that we had many corresponding experiences. The young Martin cycled around town to his first small telescopes and astronomy club meetings; I rode the New York subway to do the same. He twice mistook an internal reflection for a new comet; I watched a student repeatedly "adjust focus" of a Kitt Peak telescope by tweaking what was in fact the counterweight balance adjustment. We both erected mid-sized telescopes in the mountains near Tucson, and apparently both of us found that large, lightweight aluminium mirrors don't work, regardless of what experts once wrote.

In Quest of Telescopes aims to show high-school students and others what the astronomer's life is like. This is accomplished as much by the brief accounts of how major insights and discoveries were achieved as in the tales of events in Minnesota, Arizona, Chile, California and Hawaii. The book provides a fine description of what it is like to visit and work at a great national observatory. There also are many instructive anecdotes, interspersed with amusing tales fit for telling on cloudy nights while the wind whistles round the dome. Of these, the best is Cohen's recollection of the invasion of an infrared observatory by a horde of moths, who so filled the optical path of the telescope that it was possible to sense their temperature if not, temporarily, that of the stars. I also was glad to find descriptions of the as yet inexplicable problems that intrigue the author, such as the circumstance that large and small stars seemingly are born in separate nurseries. Perhaps a young reader will some day find the answer.

This *Quest* lacks an account of teaching, which is a major responsibility for so many astronomers. Note also that the young readers inspired by Cohen will find fewer opportunities to enjoy the observatory life than did he; an "observing run" now often means a trip to a prosaic NASA or ESA



NASA

The Space Telescope — new opportunities for astronomers.

satellite control room. Further, in the 1990s, the data from the major mountain-based telescopes will surely flow to central city terminals or even directly to the "observer's" home institution, and jet-setting will fall off accordingly. High above, however, a few lucky astronomers will be operating their telescopes aboard the Space Shuttle. □

Stephen P. Maran, a Senior Staff Scientist in the Laboratory for Astronomy and Solar Physics at the Goddard Space Flight Center, is currently helping to build a spectrograph for the Space Telescope.

The chemists' Earth

Peter J. Smith

The Earth: Its Birth & Growth. By Minoru Ozima. Pp.117. ISBN hbk 0-521-23500-6; ISBN pbk 0-521-28005-2. (Cambridge University Press: 1981.) Hbk £10.50, $22.50; pbk £3.95, $8.95.

HERE is a cautionary tale. In 1947 the research vessel *Atlantis* dredged some metamorphosed basalts from the mid-Atlantic ridge at 30°N. When these rocks later came to be dated by the potassium–argon method they were found to be about 48 million years old, far from the roughly zero age they should have had on the basis of the seafloor spreading hypothesis just becoming popular. At the time this little inconsistency was put down to "error" (possibly the presence of excess argon-40, a well known age-invalidating phenomenon) and then conveniently forgotten.

Or rather it was forgotten by almost everyone but Minoru Ozima, who in the late 1960s had another go at dating the offending samples, this time using the new argon–argon technique with its isochron consistency test. In this way the correct age of the basalt was found to be 170 million years, thus producing even greater consternation involving accusations of specimen mishandling and cries of triumph from the "anti-spreaders". Observation and hypothesis were neatly reconciled in the end, however, by concluding that the basalt must have been left behind at the ridge from the time of the initial split of South America and Africa.

Only a geochemist would have the gall to embarrass the geophysical gods by dragging up this sort of thing; but the simple moral of the anecdote is well taken, namely, that isotope geochemistry has a vital role to play in elucidating the details of plate tectonic processes. What is more, unlike plate tectonic studies, which have hitherto concentrated largely on the past 200 million years, geochemical investigations are crucial in interpreting the whole course of the Earth's evolution. Earth scientists know this already, of course; but for the less specialist reader the more sober geochemical story has tended to fade against the background of the past two decades' blaze of geophysical publicity.

Ozima has set out to correct this unjust imbalance by providing for the general reader a short account of the way in which natural isotope studies have been used to trace the Earth's broad development. Beginning with the "birth of elements" in the nucleosynthesis that preceded the formation of the Solar System, he then looks in turn at the accretion of the Earth as a whole, the formation of the core, the separation of crust and mantle, subsequent changes in the crust, and the origin of the oceans and the atmosphere. The book ends with a discussion of the ways in which man-

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made, high-level radioactive wastes might be disposed of in the Earth — a nice irony considering that the sites under consideration have been shaped almost entirely by the heat from radioactive isotopes, albeit different ones.

To a great extent, isotope geochemistry is a matter of dating events and processes that have self-evidently taken place or have been shown to do so using other types of evidence. This has tended to give the topic a "service industry" image, which is perhaps another reason why would-be popularizers have concluded that it lacks glamour. Ozima's considerable achievement in this book has been to demonstrate to laypeople and students the vitality, excitement and importance of isotope geochemistry as a subject in its own right. In this cause he has been helped beyond measure by Judy Wakabayashi, who has produced a beautiful translation that puts the majority of native scientific English writers to shame. □

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Ultimate histories and final destinations

John Noble Wilford

Cosmic Dawn: The Origins of Matter and Life. By Eric Chaisson. Pp.302. ISBN 0-316-13590-9. (Atlantic Monthly Press/Little, Brown, Toronto: 1981.) $14.95. *Genesis: The Origins of Man and the Universe.* By John Gribbin. Pp.360. ISBN UK 0-460-04505-9; ISBN US 0-440-2832-9. (Dent/Delacorte: 1981.) £7.95, $13.95.

IN content, purpose and intended audience (the educated layperson), these two books are much alike. Both cover the same ground, which is to say that they start with the big bang and bring us forward through the creation of the Solar System to the emergence of life and the ascent of man. But they differ considerably in style. *Cosmic Dawn* is an extended essay, instructive and literate, building toward a moving statement on the human condition and prospects. *Genesis* is a comprehensive exposition of current consensus and debate on our origins, tutorial in style but lively and often provocative.

Chaisson, an associate professor of astrophysics at Harvard, has produced a work of history as much as of science, with its narrative force and synthesis of grand themes. Astrophysicists, as he says, are the "ultimate historians". His essay is shaped around two "critically important transformations in the history of the universe". The first was the emergence of matter from radiation; the second, the emergence of technologically intelligent life.

The latter, he writes,

> is the quintessential event in the development of matter, the threshold beyond which life forms can truly begin to fathom their role in the cosmos. Significantly, then, we have an obligation, a responsiblity to survive.

To survive as a civilization Chaisson contends that we must circumvent the very real possibilities of overpopulation, self-destruction, genetic degeneration and subjugation by computers. Long-term survival of the species, he states in an eloquent conclusion, may depend on our commitment to a two-fold programme of simultaneously colonizing the nearby planets, which he sees as a dispersal of the species so as to be less vulnerable to local catastrophe, and searching for galactic civilizations, which, if found, might reassure us that others have attained technological power and still avoided doomsday. The case for extraterrestrial exploration has rarely been better stated.

Gribbin, an astrophysicist and prolific science writer, also concludes with a forward-looking chapter, "Destinations", and suggests, among other things, the possibility of our descendants developing a "space culture". But, in general, his discussion of the future is unfocused and unsatisfying. This is a pity, for the rest of the book is so clear and rich in detail and insight. Much more than Chaisson, Gribbin spells out the discoveries and reasoning behind current thinking in cosmology and biological evolution. His discussions of plate tectonics, the origins of diversity and the origins of Earth are especially lucid. His style, like a gifted lecturer's, is by turns playful and authoritative. The index and organization by subheadings make it easy to refer back to subjects of particular interest.

Either book can be recommended to the student who seeks a broad understanding of the origins of mankind and the Universe (students of the humanities might prefer *Cosmic Dawn*), or to the general reader who wants a rewarding experience delving into the "ultimate" history. □

John Noble Wilford is a science correspondent of The New York Times *who specializes in space exploration. His most recent book is* The Mapmakers *(Knopf, 1981).*

Michael Ruse's *The Darwinian Revolution* (reviewed in *Nature* **284**, 670; 1980) has been published in paperback by Chicago University Press, price $9.95 (US only).

Pressure to scale

W.D. Hackmann

Barometers. By Bert Bolle. Pp.256. ISBN 0-85242-710-7. (Argus, Watford, Herts: 1981.) £12.50. *Early Scientific Instruments.* By Nigel Hawkes. Pp.168. ISBN 0-89659-192-1. (Abbeville Press, New York: 1981.) $29.95.

COLLECTORS of antiques are becoming increasingly interested in scientific instruments. Sundials, telescopes, microscopes and barometers are amongst the most popular. Barometers, especially, have commanded wide interest because of their attractiveness as pieces of furniture. Bert Bolle, a Dutch antique-dealer, has been a barometer enthusiast for many years. His book, which was first published in The Netherlands in 1978, occupies a niche between the purely technical histories, such as that written by W.E.K. Middleton in his *The History of the Barometer* (Johns Hopkins Press, 1968), and those describing antique barometers, of which the best English example is N. Goodison's *English Barometers 1680–1860* (Cassell, 1977).

The book is divided into three parts. The first describes the evolution of the various barometer types, and each chapter deals with a specific pattern: stick, marine, miner's or pit, sign-post, banjo and aneroid. Ancillary instruments, such as thermometers, hygrometers and psychrometers, are also discussed briefly. Between 70 and 80 thermometer scales were in existence in the eighteenth century, and a Dutch example of 1754 with 18 scales is illustrated.

The earliest barometers were simple tubes containing mercury, based on Torricelli's well-known experiment of 1644, to which Descartes added a scale four years later. At first, the arrangement was used to demonstrate the "weight of air", but there was a slow realization that it could be used in forecasting weather. Research shifted to England. Both Robert Boyle and Robert Hooke improved the design, and gradually during the 1680s instrument-makers, clock-makers and opticians started to manufacture barometers for domestic use. Within a short period the main mercury barometer types were established. Trade concentrated in Britain, and to a lesser extent in The Netherlands, where a double or contra tube system (the contra-"bakbarometer") was invented by Huygens. Amongst the earliest makers were the famous clock-makers Thomas Tompion and Daniel Quare, then, during the eighteenth century, the manufacture of barometers became the speciality of expatriate Italian craftsmen; two of the best-known Victorian firms, Negretti & Zambra and Pastorelli & Rapkin, evolved in this way. In The Netherlands, too, Italian makers appeared in the 1750s.

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The second part of the book is an illustrated catalogue of about 135 barometers. As expected, most examples are British, but France, Germany, The Netherlands, Denmark and Austria are also represented. Finally the author deals with the barometers and ancillary instruments encountered in the preceding parts from a technical point of view. Useful tables converting old into present-day units are given, and also recipes, for example for the camphor solution used in storm glasses. A novel arrangement of the book is that in each of the three parts the same chapter numbering is used when describing the same class of barometer. For instance, Chapter 3 in all three sections refers to the stick barometer. This facilitates cross-referencing, but also causes some duplication.

Michael Holford's beautiful photographs give the second book its obvious appeal. Nigel Hawkes, the science correspondent of *The Observer*, has been eclectic in his selection, ranging from a sixteenth-century astrolabe to a late nineteenth-century mahogany and brass telephone switchboard. The instruments can be seen in three London institutions: the Science Museum, the National Maritime Museum at Greenwich and the Royal Institution.

A remarkable feature of the book is the extraordinary size of the lettering, usually associated with books specially printed for the elderly suffering from myopia. No doubt this was necessary so that the somewhat bare text could fill the large page size required for the plates. The descriptions given are reasonably accurate, indeed in a few instances more correct than the museum labels exhibited with the original instruments; but this is above all a picture book, which gives only haphazard glimpses into a fascinating history. □

W.D. Hackmann is Assistant Curator of the Museum of the History of Science, University of Oxford.

History through the looking glasses

Silvio A. Bedini

Collecting Microscopes. By Gerard L'E. Turner. Pp.120. ISBN UK 0-289-70882-6; ISBN US 0-8317-5950-X. (Studio Vista, London/Mayflower, New York: 1981.) £2.95, $14.95. *The Camera Obscura: A Chronicle.* By John H. Hammond. Pp.182. ISBN 0-85274-451-X. (Adam Hilger, Bristol/Heyden, Philadelphia: 1981.) £13.50, $33.50.

As Gerard Turner states in his introduction to *Collecting Microscopes*, "A book for collectors of microscopes must be both a history book and a guide book". It must not only describe the instrument's development, but also identify the leading makers and the capacity of the instrument in its various forms to perform its scientific tasks. This is indeed a large order for a volume of modest size, but the author manages to meet his own requirements extremely well.

The microscope has had a long and complex history in evolving from the simple instrument of the early seventeenth century to the modern electron microscope. It was an endeavour in which many countries took part and to which its users — anatomists and other men of science — made as substantial contributions as did the makers of the instruments. It is not surprising that in time the microscope became the universally recognized symbol of science because it had such a wide range of application, from medicine to geology and from botany to metallurgy.

The book ranges from descriptions of the optical structure of the microscope, the anatomy and materials, to individual chapters on the several basic structural forms. Special attention is given to the English and Continental microscopes produced in the nineteenth century, the period which saw its greatest development. Noteworthy are sections on special hints to collectors, names and addresses of the most important museum collections throughout the world, a guide to prices based on auction and other recent sales, and a comprehensive bibliography. The work is illustrated by splendid pictures, many in colour, and by numerous diagrams.

The author is recognized as one of the world's foremost authorities on the history of the microscope, and is the author of many writings on the subject. His early training as a research physicist and his museum experience contribute greatly to this attractive, authoritative volume, which no collector of microscopes, historian of science or museum curator can afford to overlook.

Hammond's history of the camera obscura is also a welcome addition to the literature of optical instruments; again, it will serve not only the collector but the historian as well. In this informative volume, Hammond traces the history of the instrument from its use by fifth-century Chinese philosophers through Greece to Europe, where it was known by the thirteenth century.

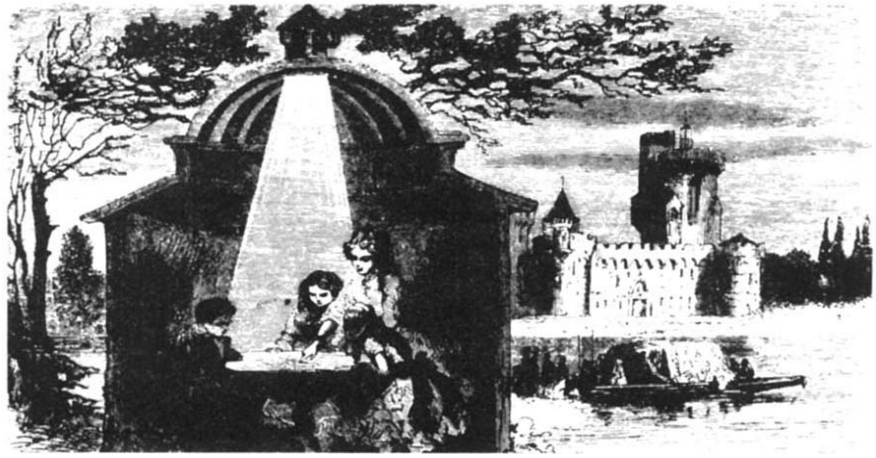
Simply stated, a camera obscura is a darkened room or an enclosed box into which light passes, generally through a lens, to form an image of external objects on the opposite surface. Gemma Frisius, Nicolas Copernicus, Tycho Brahe, Moestlin, Johannes Fabricius and Johann Kepler were early users of the instrument for observing eclipses, sun spots and planetary movements. The first portable model appears to have been designed by Robert Boyle and it was immediately imitated by others. Kepler devised a portable form in a tent which he probably used for his survey of upper Austria as well as for observing astronomical phenomena.

The camera obscura progressed from serious scientific — and artistic — use to a device for public entertainment by magicians and other performers, and eventually was reduced to a parlour diversion. It was the subject of an extensive literature during the period of its maximum popularity, but more recently it has merited little more than passing mention in histories of science, art and photography.

In addition to a lucidly written, well-illustrated history, Hammond provides appendices and lists of references at the end of each chapter, as well as a comprehensive bibliography and full index.

Both of these authors have succeeded admirably in their aims; their books are contributions to the scientific literature as well as most useful and attractive guides for the collector. □

Silvio A. Bedini is Keeper of Rare Books at the Smithsonian Institution, Washington DC, and specializes in the history of scientific instrumentation and horology.



From *The Camera Obscura*, courtesy Science Museum, London.

The camera obscura as a diversion for children. The picture comes from F. Marion's *Wonders of Optics* (1868).

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Entry to the history and philosophy of science and medicine

Donald Cardwell

Dictionary of the History of Science. Edited by W.F. Bynum, E.J. Browne and Roy Porter. Pp.496. ISBN UK 0-333-29316-9; ISBN US 0-691-08287-1. (Macmillan Reference Books, London/Princeton University Press: 1981.) £17.50, $40.

THE lexicographer is, according to a high authority, a drudge. But this description hardly fits the three editors of the *Dictionary of the History of Science.* All are active scholars in the history of medicine and they have had the assistance of no fewer than ten subject editors. The title of their dictionary, however, requires amplification. What we are given is, in fact, an encyclopaedia of the history and philosophy of science, comprising short articles on general topics of between 50 and 2,000 words, interspersed between about three times as many brief references to specific points in the short articles which, in effect, constitute an index. The form of the work is, then, a cross between an encyclopaedia and a dictionary with the emphasis on the former. Few archaic or obsolete words are included; alembic, athanor, pelican and that legion of pre-scientific chemical terms are absent.

In short, this dictionary is more didactic than narrowly informative and it reflects a very definite view of the history of science. Technology is confined to one article while the philosophy of science and the history of medicine are well represented. This policy must have entailed problems for the editors — barometer, cloud-chamber, galvanometer, thermometer all qualify for inclusion, while ammeter, voltmeter, bridge (electrical) do not. Presumably the latter were considered technological and therefore outside the scope of the *Dictionary*. Clearly, there is a need for a companion volume on the history of technology.

A perusal of the brief references is not without its lighter moments. Anyone who is not a demographer must be intrigued by the reference: "average man. *See* statistics (vital)". On the other hand, "improper egg. *See* generation" calls to mind an entry in that incomparable index to Wyndham Lewis and Charles Lee's anthology, *The Stuffed Owl,* "Eggs, mention of, wrapped in elegant obscurity, 62". The reference, "women. *See* chlorosis; hygiene; male–female differences" will annoy militant feminists as well as invite comparison with another entry in the aforementioned index, "Woman, useful as a protection against lions, 118". As for "museums. *See* fossils", this suggests a particularly sour opinion of museum curators.

On a more serious note, however, I must take issue with the editors on a number of points. Some of these are minor and amount to little more than expressions of opinion. Surely, for example, Newtonianism merits more than 13 lines? And should not mention of Gibbs and Heaviside be included in any account, however short, of the history of vectors? The British Association is, very properly, included but not the older Deutsches Naturforschers Versammlung (1822). Again, hospitals find a place but there is, unaccountably, no room for laboratories. And for their part, Mancunians must regret that no reference is made to daltonism.

Most serious, in my view, is the failure to mention units and dimensions. The development of the theory of dimensions and the establishment of rational units were, taken together, a most important feature of the growth of physics in the past 200 years. The British Association Committee on electrical units (1861) may have been a response to the needs of telegraph engineers and the results of its labour may have been vital for the establishment of the world's electrical supply industry, but they were hardly less significant in the realm of "pure" science, in the formulation of electromagnetic field theory. After all, Maxwell was a member of the Committee and his seminal paper was published in 1865.

On the other hand, when we consider the articles that are included we are amply compensated. Apart from a few brief passages about contemporary and fashionable theories of science, that may well prove ephemeral, the short essays that form the bulk of the *Dictionary* cover an enormous variety of subjects, ancient and modern. These range from Ptolemaic astronomy and Islamic science to matrix mechanics and causality in quantum physics, from Aristotle's cosmology to the paradox of confirmation, from *Naturphilosophie* to molecular biology and the genetic code. The *Dictionary*, in other words, provides material for a comprehensive course on the history and philosophy of science.

The omissions can easily be attended to in future editions and, all things considered, this is a valuable work. It is well produced and the individual articles, by nearly 100 scholars, are authoritatively, carefully and clearly written. Every library and every scientific establishment should have a copy. □

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Plants in print: more of Thornton's *Flora*

S.M. Walters

The Temple of Flora. Introduction by Ronald King. Pp.112. ISBN UK 0-297-77984-2; ISBN US 0-8212-1128-5. (Weidenfeld & Nicolson, London/New York Graphic Society, Boston: 1981.) £18.50, $35.

THE spate of handsomely produced botanical and horticultural books continues unabated, in spite of the recession, and even professionals find it difficult to pick their way through them. A new edition of Robert Thornton's *Temple of Flora,* containing a complete set of reproductions of the famous colour engravings, is, however, a publishing event overshadowing most of the rest — especially at such a remarkably modest price. In a detailed introductory section, Ronald King, a former Secretary of the Royal Botanic Gardens at Kew, tells the story of this extraordinarily grandiose venture by Thornton, who dissipated a private fortune on his scheme in the first years of the nineteenth century. King's introduction goes further back than the reader might expect, beginning with the birth of Linnaeus, and tracing the rise of Linnaean botany in the second half of the eighteenth century and its relation to botany and horticulture as relaxations and hobbies enjoyed by Queen and humble citizen alike.

Thornton received an important part of his education in Cambridge, where he obtained his medical degree in 1793, and came under the influence of Thomas Martyn, Professor of Botany, a great supporter of the new Linnaean systematics. His career in medicine seems to have been both unorthodox and relatively unsuccessful. The evidence is that he had conceived something of his grand plan for a magnificent book on Linnaean botany as early as 1791, whilst still at Cambridge in fact, although it was six years before his circumstances enabled him to launch the project. The inglorious failure of the whole scheme in the "Royal Botanic Lottery" of May 1813 is described in detail; it is a quite extraordinary story, from which Thornton emerges as a figure to be pitied or despised according to taste. He was never to know that the third volume of his great work would achieve permanent and international fame — a "lasting heirloom for the British nation", as King says.

The generous layout of this book involves some repetition, particularly between the text accompanying each plate and the relevant page in the Introduction, and the reader should be warned that

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important information about the plates may only be found in the introductory reference. As an example, the botanical identification of the plates, a technical and specialist matter, is not systematically included in the legends, although it can (in all cases?) be gleaned somewhere in the Introduction. Proof-reading seems to have been very good, though a small error occurs on p.16 in the title of Sir John Hill's *Flora,* which is given as *Flora Botanica* — a particularly odd mistake, since Sir John himself had mis-spelt his own title by calling the work *Flora Britanica.*

An adequate bibliography provides access to all relevant works including the most recent (1972) edition of the *Temple of Flora,* published by Collins and now out of print. Purchasers of this new edition may find it interesting and instructive to consult the Collins book in a library; in it Geoffrey Grigson gives a more concise account of Thornton's work which is also less tolerant of his vanity, greed for success, taste for bad poetry and generally (to us) his excessively "purple" prose style than is that given by King. Yet both agree, as the whole botanical world does, that the plates are superb. The publishers are to be congratulated on making them available again. □

S.M. Walters is Director of the University Botanic Garden, Cambridge, and author of The Shaping of Cambridge Botany *(Cambridge University Press, 1981).*

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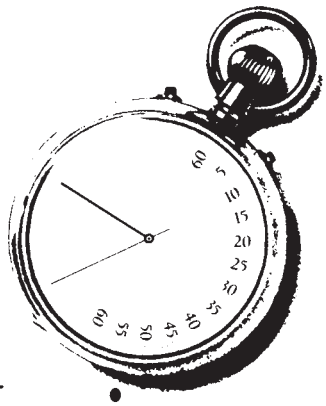

Keeping pace with science

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The flowering of botanical illustration . . .

Sandra Raphael

The Art of the Botanist. (In the USA *The Art of the Plant World.*) By Martyn Rix. Pp.224. ISBN UK 0-7188-2482-2; ISBN US 0-87951-118-4. (Lutterworth, London/ Overlook Press, New York: 1981.) £25, $60 until 31 December, £30, $75 thereafter.
Curtis's Flower Garden Displayed. Descriptions by Tyler Whittle and Christopher Cook. Pp.258. ISBN 0-19-217715-X. (Oxford University Press: 1981.) £19.50, $59.

THE best botanical illustrations, most obviously the greatest eighteenth-century ones, transcend their strictly utilitarian purpose of recording the appearance of plants and become works of art in their own right. Dr Rix's book is called *The Art of the Botanist,* and its large format seems to give priority to the pictures; but the text that accompanies them is more like a frustrated account of botanical exploration than, as is intended, a history of botanical illustration. In an ideal world, this subject would be treated by an author equally at home with botany, bibliography and art history. Dr Rix is primarily a botanist, and the book reflects this lack of balance, for he is obviously more comfortable once he reaches the eighteenth century and the start of modern botany.

The first quarter of the book skims over the earliest manuscript and printed herbals and plant pictures, ending with a chapter on exploration in the sixteenth and seventeenth centuries and the books that hold the records of the new plants of the period. The next, and longest, part of the book deals mainly with the eighteenth century, perhaps the greatest age of botanical illustration, with a geographical arrangement leading to a tangled treatment of chronological developments. The final section starts as lithography begins to take over from engraving and covers nineteenth-century publications and the merest handful of twentieth-century work.

The pictures are, of course, the book's *raison d'être* — 250 of them in black and white and 64 in colour, all in only 220 large pages. They are beautiful, and the photographic quality is generally good; but the book's design does not give them the setting they deserve. The pages are packed, but the demands of colour-printing mean that pictures are often some distance away from the relevant text. No indication of the size of the originals accompanies the reproductions, so that those unacquainted with the books concerned are given no idea of their scale. Black frames surround nearly all of the pictures, a real distraction when several are printed on one large page, when the effect produced is that of a sheet of large stamps. The colour plates on pages 160 and 161 make a really unhappy pair, the strelitzia from Redouté's *Les Liliacées* — at its best a clownish plant and hardly a beauty — facing the night-blooming cereus, complete with romantic background, "moonlight by Pether", from Thornton's *Temple of Flora.* Both of these would look infinitely better with more compatible neighbours.

An advertisement for *The Art of the Botanist* has described it as a definitive history of its subject. Even if one makes allowances for the context of such a statement, it is one to be treated with great caution. The book's bibliography is absurdly inadequate. Many important sources of relevant information appear to have been ignored, not least the Calmann study of G.D. Ehret, published in 1977 and the standard work on one of the greatest of flower painters. Dr Rix says a good deal about the Duchess of Portland's collection of Ehrets, although he is apparently unaware that two volumes of them have recently been identified in Oxford. He also mentions the Windsor copy of an Aztec herbal of 1552, "in which only one or two of the plants are actually recognisable", without troubling to tell us that the Windsor herbal is apparently a copy of the Badianus manuscript in the Vatican Library, published in facsimile in 1940 by E.W. Emmart, who also provided copious notes in which several of the plants are identified. The Plantin collection of plant illustrations, the source of so many of the pictures in sixteenth- and seventeenth-century herbals, is given a few lines, but the recent rediscovery of it in Cracow, after its removal from Berlin during the last war, goes unnoticed. So one could go on. In addition there is the usual crop of minor misprints, among others, that hardy annual that turns the Pierpont Morgan Library into the Pierpoint Morgan.

Dr Rix's own travels have taken him to some of the regions explored by earlier botanists, and his book is most vivid when he can add his own first-hand comments to theirs. A member of Tournefort's expedition discovered a large vetch, apparently encountered by Dr Rix too, for he tells us that its "succulent young shoots" are "still much eaten by shepherd boys".

A picture book then, but not a definitive history of botanical illustration. For the nearest approach to such a thing we must still rely on Wilfrid Blunt's *The Art of Botanical Illustration* (1950) — much quoted by Dr Rix — and Claus Nissen's *Die Botanische Buchillustration* (1966).

Curtis's Flower Garden Displayed includes 120 plates chosen from about a thousand printed in *Curtis's Botanical Magazine* during its first 20 years (1787--1807) and 25 volumes. This periodical, now not far short of its bicentenary, was founded to describe garden and greenhouse plants, each plate being accompanied by a short note giving a

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description, a potted history and notes on cultivation. The plants selected by Tyler Whittle and Christopher Cook have been arranged in order of their introduction to Europe, with the original text replaced by a new one, mixing botany, gardening and historical chit-chat with quotations from Turner, Gerard, Parkinson, Curtis himself and other botanical classics.

"*Rhus*", possibly the stag's horn sumach (*Rhus typhina*). Woodcut from *Commentarii in Sex Libros Pedacii Dioscoridis*, 1565 edition, by Pierandrea Matthioli.

Photograph Cameron & Tayleur from *The Art of the Botanist*.

The arrangement echoes that of the *Botanical Magazine* itself, each plate facing its appropriate text. The plates are good copies of the originals, and the book is a very attractive production, making it even more of a pity that such a pleasant piece of printing is spoiled by extremely careless copy-editing and proof-reading. Misprints are numerous, perhaps the most comic being "naval wort" for navel-wort. Several personal names appear in variant forms; even Linnaeus's are given in the wrong order: Linnaeus was his family name, and he did not become von Linné until he was ennobled.

This selection of early Curtis plates will be welcomed by gardeners interested in the history of the plants they grow. It might even lead them to a full set of the *Botanical Magazine* itself, or perhaps to the dozen plates from recent issues sold as a calendar at Kew each year — a pleasant annual glimpse of some of the best of today's botanical illustration. □

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. . . and of the garden at Cambridge

Blanche Henrey

The Shaping of Cambridge Botany: A Short History of Whole-Plant Botany in Cambridge from the Time of Ray into the Present Century. By S.M. Walters. Pp.121. ISBN 0-521-23795-5. (Cambridge University Press: 1981.) £17.50, $42.50.

THIS slim quarto volume was published to mark the 150th anniversary of an Act of Parliament, dated 30 March 1831, authorizing the acquisition of land from Trinity Hall for the development of a New Botanic Garden. By that time the eighteenth-century Walkerian Garden founded in 1762 had become too small, and, in the words of the Professor of Botany, J.S. Henslow, "utterly unsuited to the demands of modern science".

The book gives a brief and interesting history of the scientific study of botany in Cambridge; and the author, though not going very deeply into his subject, provides the botanist with a useful and handy source book. Moreover, at the present time when the study of garden history has such a wide appeal, a publication with special reference to the teaching, planning and research, carried on over the years in the garden at Cambridge, offers much of interest to the garden historian.

Dr Walters, at one time Curator of the Cambridge Herbarium and Lecturer in the Botany School, and now Director of the Garden, has written this book chiefly from material obtained from published works, from some unpublished manuscripts and from knowledge that he has acquired over many years of association with the University. It consists of a history of botanical and horticultural science in Cambridge from the seventeenth century, when John Ray established the tradition of herborizing, or plant hunting expeditions, until the establishment of specialized modern branches of botany, such as genetics and ecology, in the present century.

The eight chapters cover various stages in the development of the Garden and Cambridge botany. Details are given of the lives of the important figures in the history of the science and the part that some of these men played. Among them, from the time of Ray until 1825, are Richard Bradley, and John and Thomas Martyn, and from 1725 onwards, John Steven Henslow, Charles Cardale Babington, Sydney Vines, Richard Irwin Lynch, and Harry Marshall Ward and his successors.

A chronology at the end of the work, giving dates of historical events, lives of personalities, publications and so on, would have been a useful addition. In compensation, a feature of the book is the large number of illustrations — 84 in all — depicting title-pages, frontispieces, maps, portraits, plants and other subjects. There are also illustrations of specimens from Ray's own herbarium, from Martyn's herbarium, and an illustration of an herbarium specimen *Silena maritima* made by James Donn. Furthermore, a number of figures of plants are reproduced from original drawings made specially for the work by Michael Hickey. Many of the figures occupy the wide margins of the book, enlivening the pages and creating a unity between text and illustrations. A coloured reproduction of *Rosa* "Cantabrigiensis" from a water-colour drawing by Graham Stuart Thomas, Gardens Consultant to the National Trust, makes a charming frontispiece. □

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Britain in leaf

Alan Mitchell

A SINGLE volume on trees cannot, unlike one on birds, extend its coverage to the Mediterranean shores without losing much of its use in British gardens. *The Hamlyn Guide to Trees of Britain and Europe* by C. Humphries, J.R. Press and D.A. Sutton (Hamlyn; hbk £5.95, pbk £3.95) is a good example. Of the total of 20 species of pears and eucalypts covered, 15 are not grown in Britain. To encompass these and some obscure southern oaks and maples, the authors have had to omit important trees like Low's and Nikko firs, and, worse, ignore all the cultivars of Lawson and Sawara cypresses which crowd our parks and gardens. The book, then, will be of most use in the field in southern Europe. It has a good, workable synoptic key, and excellent descriptions and illustrations of details, though the whole-tree drawings are seldom in the least helpful and a few are quite wrong.

Alan Fairhurst and Eric Soothill's *Blandford Guide to Trees of the British Countryside* (Blandford, £9.95) contains beautiful photographs of the bark, foliage, flowers and fruit of the 55 trees, 9 shrubs and ivy which are given full treatment, and of a few of the 86 trees and 18 shrubs more briefly described or just mentioned. This last, however, is quite pointless when so many common trees are omitted altogether. The marginal outline drawings are reasonably distinctive (but "Dutch elm" is plainly English elm). The strength of this book, and exceptional feature, is the long lists of fungi and insects associated with each species, and of local names for the trees. The entries for timber uses and the general information are also good value.

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A particularly handsome but unpretentious little book is *The Guinness Book of Trees* (Guinness Superlatives, £4.50). Here, Jeanette and Esmond Harris give a useful summary of the history of trees in Britain from pre-glacial times until the present, and raise a question-mark against the native status usually accorded to the beech and hornbeam. They select 18 conifer and 32 broadleaf trees for full treatment and full-page colour illustration, mostly of high quality and of fine specimens. The order in which the species are presented seems to have no rationale but there is much interesting information about each.

Unfortunately, the maps are a disaster area. That showing the areas of the world from which most exotic species are derived shades the wrong side of South America and even manages to omit California, Oregon and Washington as well as Korea; and the map of tree-collections puts Edinburgh north of Stirling and Oxford in Cambridge. The distribution maps, in bold red and white, even for exotics planted widely, are equally eccentric. Ireland is deprived of grey poplar (the largest specimens in the British Isles are there) and Norway maple, but is covered in English elm. Nonetheless, the line-drawings of foliage are neat and attractive and the book should attract its fair share of buyers.

The International Book of the Forest (Mitchell Beazley/Simon & Schuster; £14.95, $35) is one of those books of large format and prolific illustration in which information from the text is repeated in boxes and captions, as if to cater for those who cannot face reading the whole tome. The scope in subject, time and region is enormous — from Pangaea through mediaeval forests to modern logging; birds, nocturnal creatures and forest man, to fire and the ecosystem, a single, double-page spread for each. Forest products also take a spread each but while elaborate maps and tables of the world timber trade may have their uses, the details of boat-building and of the manufacture of rubber tyres and plastics are out of place in a book on forests which can spare only a small box for the subject of tree-breeding. The main feature of the book is the surveys of forest types of the world, including the oceanic islands and mangrove swamps, and these include some magnificent photographs.

In complete contrast, as befits its different function, is *The Reader's Digest Field Guide to the Trees and Shrubs of Britain* (Reader's Digest Association, £6.50), a stoutly bound, attractive book in an unusual broad, shallow format. It is profusely illustrated with mostly good paintings and drawings, and one high-quality photograph per page, which last is, however, too small to be of much value. Arranged according to the shape of the leaf, the plants include a wide range of shrubs and yet there is still room for a good selection of trees and some cultivars. A broad ecological slant is given by additional coverage of the wildlife in oak woods, beech woods and other forest types. There are five spreads depicting winter shoots and two on cones, and a small section on some of the places in which to see a variety of trees. Produced to the usual standards of Reader's Digest books, this is a good specimen of the field-guide genre and a reasonable buy. □

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Arboreal splendours and uses

Richard Howard

The Oxford Encyclopedia of Trees of the World. Consultant editor Bayard Hora. Pp.288. ISBN 0-19-217712-5. (Oxford University Press: 1981.) £12.50, $19.95.

"ASK a child to draw a tree and he will start with the trunk, then add a fan or brush of branches". A straight, woody, self-supporting trunk, unbranched for a distance from the base, defines a tree at least 21 feet in height to most people. Yet the botanist or the horticulturist may be uneasy with such a definition, which in this book covers ferns (to 80 feet tall), cycads, grasses (bamboo), dragon trees (supposedly 6,000 years old), tree cacti (to 66 feet), as well as the broadleaved flowering plants and the cone-bearing gymnosperms with needle-like foliage. Trees in cultivation, the primary subjects of this volume, have a history as well as individual beauty and are useful to mankind for their bark, wood, leaves, flowers or fruit.

Thirty-nine authors excel in describing, distinguishing and illustrating the 350 species covered in this volume. The "main" species of large genera are grouped together, with notes on their key characteristics, permitting the reader to identify a plant and to understand the morphological variation within the genus. Useful keys for the identification of trees are provided for conifer families and the conifer genera by families, for the broad-leaved families and aberrant genera, and for the broadleaved genera by families for those plants covered in the book. The introductory essays on trunk and wood structure, the forest ecosystem, the forests of the world, and forestry practices and products are instructive evaluations of the economic problems faced by a society which is still dependent on the tree.

The photographs, maps of distribution and drawings are well done, though there are a few exceptions. The photograph of *Cornus kousa* is a disappointing representation of that handsome flowering tree, and no one would understand the attraction of the American flowering dogwood or the Pacific dogwood from the short, leafy branches used as illustrations here.

The volume is not an "encyclopedia" of trees of the world, as titled, but is certainly a handsome compilation of "trees of many kinds, primarily those grown in Europe". The treatment of the gymnosperms (conifers) is the best ever published and alone is worth the price of the book. In contrast, the section "Trees of the Tropics" is atrocious, with errors of fact (e.g. mace, *Bertholletia*), identification (plate of jackfruit, text on *Hura crepitans*), and omissions of interesting or important trees (litchi, *Blighia*), and with a vocabulary inconsistent with the glossary or the other articles. Fortunately it is short and, if ignored, will not detract from what is otherwise an excellent publication. □

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Anatomy of biology

Peter R. Scott

Biology in Profile: A Guide to the Many Branches of Biology. Edited by P.N. Campbell. Pp.128. ISBN hbk 0-08-026846-3; ISBN pbk 0-08-026845-5. (Pergamon: 1981.) Hbk £8, $19; pbk £3.95, $9.50.

WHAT should you tell an intelligent 17-year-old about the biological sciences? How can you convey something of the range of the subject to a bright school-child faced with the many different courses which further education offers? According to the International Council of Science Unions, the proliferation of the biological sciences has now made this task too difficult for any individual to tackle. They have therefore asked a number of distinguished biologists to explain something of their own areas of specialization; the result is this collection of 20 essays. The subjects covered may all be studied at university level, and range from zoology and botany to the less-familiar psychology, endocrinology and biophysics.

The authors were asked to address themselves to intelligent 17-year-olds who are already studying biology. There is no attempt at any consistency of style; some have needed to explain the background to their subject in detail, others have chosen

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to relate some of the more spectacular discoveries of the past 20 years. Many give their own personal view of where the subject might lead in the future, and what it might offer to those who follow it.

A brief essay is not a suitable format for a careful weighing of complex arguments; rather it provides an opportunity for an optimistic and sweeping review of a field. Most of the authors have responded with a light approach — nutrition is sold as a growth area — and this makes *Biology in Profile* suitable reading matter for spare moments. Many of the contributions assume little biological knowledge, and would also be appropriate for those thinking of taking up biology for the first time.

The treatment of biology as 20 disparate subjects may cause concern to some teachers; but overall the book does not create an impression of fragmentation. None of the authors is able to describe his own area without referring to its overlap with topics described elsewhere — indeed, the idyllic picture is drawn of scientific progress being made by the combined efforts of biologists, chemists and physicists, all working in close cooperation. Some students may be surprised to see the emphasis placed by many of the writers on quantitative work; those who see biology as the only science which does not require a firm grasp of mathematics would be well advised to choose their area of specialization carefully.

Perhaps the most surprising omission from this collection is any discussion of medicine or veterinary science. Most of those who enjoy biology at school will consider these as a career, if only because they imagine that they know what they entail. A direct comparison with the other branches of biology might have been helpful, and have removed some misconceptions.

Biology in Profile will find a place in many school libraries. It will be bought for those bewildered by the range of biological subjects available at university, but I think it should also be read by teachers seeking answers to the question "What is the point of science?", and even by non-biologists. May we hope for chemistry and physics in profile? □

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Lightfinger

Following the success of the 1980 production, the Natural History Museum in London will this year stage "Lightfinger: A Space Fantasy for Christmas". The "fantasy" is designed for family audiences and takes the form of a science fiction comedy dealing with inter-galactic conservation and ecological problems.

The play will run from 18 December to 21 January (excluding 24-26 December and 1 and 11 January) with performances at 14.30 from Monday to Saturday, and at 15.00 on Sundays; special matinees for school parties start at 11.00.

Elliott Coues, the combative naturalist

Clayton M. White

Elliott Coues: Naturalist and Frontier Historian. By Paul Russell Cutright and Michael J. Brodhead. Pp.509. ISBN 0-252-00802-2. (University of Illinois Press: 1981.) $28.50, £19.95.

Elliott Coues — lover of birds yet sparrow hawk.

"A MAN with the mental calibre of Huxley and the charming personality of Mivart" — thus did Alfred Russel Wallace characterize the American ornithologist Elliott Coues; and Wallace's assessment was by no means unique. Also known for his contributions as a naturalist and frontier historian, Coues' achievements were recognized during his life (1842–1899) and he enjoyed ample publicity. The memory of his work has of course faded over the years, but this account of his life will do much to bring him back into the public eye.

Cutright and Brodhead have sifted a vast collection of hitherto unpublished material in the course of compiling their excellent biography. Capturing Coues in all his diversity, this book is for everyone; it is easy reading, yet informative and — not least — entertaining. Its 26 short chapters, refreshingly error free, draw heavily on the voluminous file of Coues' letters and his personal "Book of Dates". Each focuses on significant events in Coues' life: his time in Fort Randall (Dakota Territory), the expedition along the 49th parallel, the formation of the American Ornithologists' Union (Coues was one of the three founding fathers) and so on. Additionally, we are supplied with appendices and a bibliography of Coues' work.

Elliott Coues was an indefatigable worker and writer. His scientific career began at age 20, with a summer's excursion to the coast of Labrador. Funded by the Smithsonian Institution, he billed them the remarkable total of $218.72 to cover his entire summer's expenses. Three years later, as the result of work in the American south-west, he was firmly established as a naturalist; from that point his career was a steady upward climb to the apex of nineteenth-century American ornithology. He joined or was elected honorary member of 24 learned societies, among them the National Academy of Sciences, where, at 34, he was the youngest person to become a member.

His first published article appeared in 1861, even before his Labrador work; from then until his death he poured out more than 700 formal articles, edited works and other contributions ranging through science and medicine to theosophy. His literary skills matched his writing speed. His command of vocabulary, fertile imagination, ability to weave his exceptional observational acuity into the delightful fabric of his sentences and unexpected flashes of humour became trademarks of his work. To him, orioles were "gleaming through the sombre foliage like tiny meteors", whilst bushtits were "droll folk, quite innocent of dignity, superior to the trammels of decorum". Charles Lummis commented of Coues that

> such a man never would have dried up to the proverbial scientific mummy. He humanized whatever he did without sacrifice of exactness. No one surpasses him in esoteric equipment; and as a 'readable' scientist, he was easily unrivalled.

While his prose would not be acceptable amid the stuffy curtness of our present scientific writing, I found it delightful:

> We remember the 'rift within the lute'; in the canyon wren we have the lute within the rift — a curious little animated music-box, utterly insignificant in size and appearance, yet fit to make the welkin ring with glee.

And some passages cannot but elicit a chuckle. For weeks on end the scientific expedition along the 49th parallel had no fuel but buffalo dung for cooking and warmth. But Coues' pen was equal to the functionality of the dung:

> as an agent in the progress of civilization . . . the buffalo chip rises to the plane of the steam engine and the electric telegraph, and acquires all the dignity which is supposed to enshroud questions of national importance or matters of political economy.

After obtaining both MD and PhD degrees, Coues was employed as an army surgeon. As such, he was often sent to remote western outposts; most of his time was spent in the pursuit of birds, however. Two of his works are directly attributable to this period: *Birds of the Northwest* and *Birds of the Colorado Valley*. Perhaps his major publication is his *Key to North American Birds,* an inspiration to such twentieth-century ornithologists as Ernest Thompson Seton and Frank M. Chapman. Of it, Seton wrote:

> Faults it had in abundance, I now know; but it

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was the first successful effort to take exact bird knowledge from the museum, and give it to the multitude, to place it within reach of all the world of those who love to hold our birds, not as skins, but as loving friends.

While birds were his main interest, he also collected other creatures. One of his prize finds was a rare western rattlesnake which he even stopped to collect while "under the untoward circumstances of a hasty retreat from hostile Indians". Although he often fought Indians, he eventually grew to champion them. While he saw what he considered defects or evils in their character, he concluded that

the Indian is neither a foreign power to be treated with, nor a wild beast to be hunted down, but a fellow-man to be reclaimed. Let us begin by calling him, that in the end we may make him, a brother.

Coues dabbled in theosophy and spiritualism. He became an exacting historian producing works on Lewis and Clark and other early western explorers, trappers and traders. But it is for his ornithological work that he will be remembered. His help to and influence upon Louis Agassiz Fuertes, undoubtedly America's greatest bird artist, is incalculable. And during his life Coues described 36 forms of bird and 18 forms of mammal new to science, many of them new genera and most of which are still recognized today.

Considering his intense and life-long love of birds, it is ironic that his most intense and persistent effort was an unrelenting war against one: the European house sparrow, recently introduced into North America. This particular bird he deeply loathed, and from the 1860s onward attacked it and its defenders with equal venom. It is doubly ironic that the main defender and thus combatant was Coues' friend, T.M. Brewer. Brewer was not spared abuse even after his death — "We . . . know that Brewer was a cantankerous old ass at the time he had the good taste to fall asleep in Jesus". Nor was Coues' work ineffective; eventually he was even accused of "treason" due to having "incited a riot" against the sparrow. But he yielded to neither the bird nor its friends; in 1878 he publically divided the bird's defenders into five categories, four of them composed of idiots and the fifth of the weak-minded.

In the long term, of course, his efforts were in vain; the sparrow has spread in North America from coast to coast and from central Canada to southern Mexico. And the final irony? Coues certainly would not have been pleased when, in 1975, the American Ornithologists' Union presented their most prestigious recognition, the Elliott Coues Award, to a team of researchers for their notable study on the evolution of the house sparrow in North America. □

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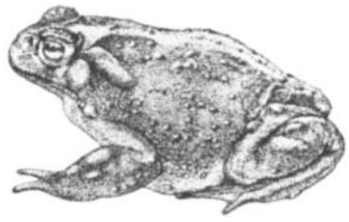
What's that (North American) animal?

Richard G. Van Gelder

The Audubon Society Field Guide to North American Mammals. By John O. Whitaker, Jr. Pp.745. ISBN 0-394-50762-2. (Knopf: 1981.) $11.95. *Harper & Row's Complete Field Guide to North American Wildlife. Eastern Edition* assembled by Henry Hill Collins, Jr. Pp.714. ISBN 0-690-01969-6. *Western Edition* assembled by Jay Ellis Ransom. Pp.809. ISBN 0-690-01971-8. (Harper & Row: 1981.) $12.95, £9.50 per volume.

THE purpose of a field guide is to enable one to identify an object without having that object in hand. This usually means that the criteria for identification are different from those on which the strict classification of the species is based. Nonetheless, some eminently successful field guides have been produced — birds, in particular, lend themselves to adequate field identification, to some extent because birds characterize themselves by sight and sound, two better-developed human senses. When it comes to animals with a greater dependence on odour or ultrasonics, such as shrews, rodents or bats, field identification becomes much more difficult. Despite the relative success of the "Peterson system" of field identification — drawings or paintings with "key" characteristics indicated, arranged for ready comparison — innovative attempts continue to be produced.

John O. Whitaker's *Audubon Society Field Guide to North American Mammals* is small enough to fit into a large pocket and is the first major attempt to utilize colour photographs for mammal identification. The 313 photographs (representing the 368 species, exclusive of whales and dolphins) are grouped rather sensibly by similar-sized and similar-appearing species, even if unrelated; for instance muskrats, beavers and marmots appear together. While this may disconcert the taxonomist, it will probably aid the layman. The colour plates are followed by 403 pages of text dealing with each species, arranged in standard taxonomic order. These accounts include a description with italicized identifying characters and measurements, sign, breeding habits, habitat, range (with a small map) and excellent though concise life-history information. Marginalia often include drawings of animals not otherwise illustrated, and tracks. Appendices include a glossary and a complex "Range Chart" to aid in the identification of some of the smaller mammals. The book is indexed and cross-referenced, but there is no bibliography.



Harper & Row's Complete Field Guide to North American Wildlife attempts to provide notes on and a source for the identification of birds, mammals, reptiles, amphibians, fish, and molluscs and other marine invertebrates of America north of Mexico. The two volumes cover the eastern and western USA, divided at the 100th meridian. This involves considerable duplication of species accounts as well as of illustrations, but not necessarily of

information — westerners are largely deprived of notes on the reproduction of mammals, for example. Within each species account there is a description, a comparison with similar species, and sadly variable and inconsistent information on habitat, habits, voice, food and range. Half of the total of 239 plates are in colour, and all are cross-referenced to the text (not always correctly or in the same volume), and "edge-marked". References are given in a section introducing each major taxonomic category.

Comparing the mammal sections, neither of these guides is superior for field identification to Burt and Grossenheider's volume, *A Field Guide to the Mammals,* in the "Peterson series", which has finer illustrations and maps. With Whitaker's book one is more likely to come up with a valid identification than with the Harper and Row volumes, but for most of the smaller species one has to resort to text descriptions and geographical information in both of these sources. Whitaker's small maps (without state or province boundaries) are useful, but the reader must be a good geographer to grasp the range of, for example, the Gray Myotis ("in se. U.S., from Ky., w. to Mo. and Kans., s. through Ala.") in a Harper and Row volume; the only map, on the inside of the front cover, does not identify the states.

For life-history information, Whitaker's book is the best single, concise source currently available, and for this reason is highly recommended. The larger Harper and Row volumes are a bit much for a pocket, and seem to have been planned by a committee to attract everyone, but satisfy no one. □

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No sense and nonsense and noses

Bryan D. Turner

The Doomsday Book of Animals: A Natural History of Vanished Species. By David Day. Pp. 288. ISBN UK 0-85223-183-0; ISBN US 0-670-27987-0. (Ebury Press/Viking: 1981.) £14.95, $40. *After Man: A Zoology of the Future.* By Dougal Dixon. Pp.124 ISBN UK 0-246-11577-7; ISBN US 0-312-01163-6. (Granada/St Martin's Press: 1981.) £8.95, $14.95. *The Snouters: Form and Life of the Rhinogrades.* By Harald Stümpke. Pp.118. ISBN pbk 0-226-77895-9. (Chicago University Press: 1981.) $4.95, £3.50.

CATS, rats, mongooses and above all mankind have caused over 300 extinctions of vertebrate animals during the past 300 years. This rate is far greater than estimates of that of the dinosaurs' extinction at the end of the Age of the Reptiles in the late Cretaceous period. David Day's *The Doomsday Book of Animals* describes something of the biology, in many cases all the known biology, of these 300 vanished species and subspecies, and chronicles their final days. The style is factual and unemotional, but the book generates a great feeling of sadness as page after page records loss after loss. Those perceptive of the value of retaining the world's biological diversity will feel something of the corporate guilt that rests on *Homo sapiens* as the destroyer of that diversity.

By far the greatest losses have been suffered by island communities, with only 75 animal species or subspecies disappearing from the continental land masses including Australia. Of these, 16 are fish which died out as their small "aquatic islands" in North America were tampered with.

Whilst the introduction of cats, rats and mongooses, whether intended or accidental, has logically resulted in the extinction of many rare endemic island species and subspecies, particularly in the West Indies and the Mascarenes, in other instances it is almost inconceivable that a species could disappear because of the sheer size of its population. The extinction of the passenger pigeon is perhaps the most astounding case since it was probably the most abundant bird species in North America. Flock sizes of between one and two million birds were reported not 50 years before the very last passenger pigeon died in Cincinatti Zoo in 1914. They were hunted relentlessly for sport, food and quack medicine, and as the flocks became more scattered so the telegraph system was used to direct the hunters' efforts. Man the hunter does not operate as do other predatory species which switch to alternatives when favoured prey are uncommon thus allowing the preferred prey to recoup its losses. Rather, he develops tunnel vision and having formed a search image is unable to switch to another prey until forced to because that prey no longer exists. It is a characteristic of the examples in the *Doomsday Book of Animals* that as a species becomes rare through man's hunting pressure, so man's efforts against that species have tended to increase. Although there may be an awakening enlightenment that the world's diversity of species needs to be conserved, David Day's beautifully illustrated and poignant book ends pessimistically by reminding its readers that there are currently over 400 critically endangered species, many of which will become extinct in the near future.

When human beings themselves become extinct and take with them many of the groups of animals we know today, new life forms will evolve to fill the resulting ecological lacunae. This is the basis of Dougal Dixon's look into the future, 50 million years hence. Although *After Man* is fun, and displays some imagination, the biological framework within which it purports to operate is clearly little understood. In many cases the futuristic species just could not follow the lifestyle attributed to them. What happens to the leaping devil (*Daemonops rotundus*), a sort of carnivorous jerboa, after it has sunk its needle teeth into a large lizard? It has neither the apparatus nor the capacity to do much else unless it swallows the lizard whole and becomes the rolling devil. How does the gigantelope (*Megalodorcas giganteus*) actually eat the bulbs and roots it grubs up with its splendid horns, which extend in front and below the animal's muzzle and prevent its getting near the ground? The book is shot through with biological anomalies and lacks credibility despite the introduction by Desmond Morris, who should have known better than to attribute the book with "real scientific value".

Whereas *After Man* tends to be conservative in its conception of alternative life forms, Harald Stümpke's (alias Gerolf Steiner) zoological fantasy is highly inventive. Absent from the bookshops for the past ten years, the reprinting of *The Snouters* has been long overdue. It is an account of the adaptive radiation of a small group of insectivorous mammals which are characterized by the extensive development of the nose or nasarium. Among the 189 species in the order Rhinogradentia the nasarium is variously used — as a temporary support while the legs serve for food processing; as an adhesive organ in the sedentary species; as a jumping organ in the hopsorrhines; and in the polyrrhines it becomes sub-divided into foot or tentacle structures. Throughout this bizarre but fascinating account the form and function are minutely examined in true Germanic taxonomic style.

Such realism will endear *The Snouters* to those with a zoological background but students should beware the rhinogrades, they are prime spoof material for inclusion in vertebrate courses. However it may be amusing to see whether straight faces can be maintained when the feeding habits of *Nasobema lyricum* are explained. This beast feeds on fruits which it reaches by, to put it politely, passing wind into its inflatable tail thus momentarily producing a long rigid appendage with a grasping tip. □

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From *After Man.*

The insectivorous flooer (*Florifacies mirabila*). The animal has glands around its mouth which produce a sweet-smelling secretion and attract the flooer's prey.

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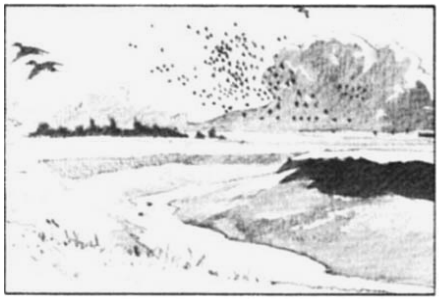
Some help for the dudes and praise to the experts

John Andrews

BIRDWATCHING beginners are badly served by publishers, their needs being met neither by the abundant field guides, which are mostly too wide-ranging and complex for the newcomer, nor by the glossy co-editions "packaged" to sell in several countries rather than to inform in one. One bright side to the recession has been that the latter have vanished from the scene, though it may be mere coincidence that two books have appeared which every beginner should own.

John Gooders' *The Bird Seeker's Guide* (André Deutsch; hbk £6.95, pbk £3.50) gives invaluable instruction on where to find birds in Britain. The opening chapters contain general guidance on the ways in which bird populations change with the seasons and on the characteristic group of species or



John Busby

"bird community" to be found in each major habitat type. The main text comprises species accounts, each containing brief identification notes which concentrate on key points for separating similar species, and a description of the preferred habitats and distributions. Where necessary for uncommon species, likely locations are given but commendable care has been taken to avoid reference to precise sites in the case of vulnerably rare birds.

Anyone venturing into the field and encountering other birders will rapidly come up against the substantial jargon of ornithology, which ranges from arcane scientific terms to offensive slang. For instance, there are about 40 different areas of a bird's plumage each with its own name and one must know them all to make clear written descriptions of birds seen in the field: if you don't, you are clearly a "casual birdwatcher without serious commitment" or, more succinctly, a dude. No longer need you flounder in ornithology's information gap. *The Birdwatcher's Dictionary* by Peter Weaver (T. & A.D. Poyser, £5) defines over 1,100 terms and abbreviations, to enable the newly-fledged birder to match insult or erudition with equal ease. Crisply illustrated by Mike Hodgson, the text is thoroughly instructive and makes good browsing as well as useful reference.

By contrast with the needs of the beginner, scientific ornithology in Britain is usually well served by carefully researched and presented books. This year has seen only a single addition to the list but one eagerly awaited by the great number of amateur ornithologists who contributed to the Birds of Estuaries Enquiry, which ran from 1969 to 1975. Every winter for those six years the total wader populations of all the estuaries of Britain and Ireland were counted in perhaps the most ambitious and demanding ornithological survey ever undertaken. Substantial organization was needed to find and coordinate the teams working each area. Few of the thousand people who took part began as skilled wader counters, so techniques had to be evolved, imparted and checked for accuracy. Some personal commitment was also required: it is no light task to rise in the January small-hours, drive to a distant estuary for a dawn high tide and strive to count whirling flocks of birds, sometimes many thousands strong, with rain-spattered lenses or frost-numbed fingers. Now the intangible and sometimes illusive rewards of the experience take a more practical form in A.J. Prater's *Estuary Birds of Britain and Ireland* (T. & A.D. Poyser, £14), which draws together the results of this mammoth study and much more information besides.

The opening chapters examine the nature of estuaries and the ways in which birds utilize them, including consideration of the factors which influence feeding success. Turning to migration and distribution in Western Europe, the author, who was the national survey organizer, discusses in particular the complexity of wader movements between estuaries which ringing work is now unravelling. Two chapters are devoted to threats to estuaries — barrages and reservoirs, airports, agriculture, energy generation, pollution and leisure all figure. This is a slightly disappointing section of an otherwise excellent work, with too much emphasis on the nature and scale of impact and too little on the causes and justification — or lack of it — for the threats themselves: some startlingly costly and grandiose ways of wasting public money continue to be dreamed up for our estuaries. Returning to his main theme, Tony Prater describes the organization of the Enquiry, discusses count methodology and considers the vexed question of assessment criteria.

About half of the text comprises detailed summaries of present knowledge, both estuary by estuary and species by species. Copious maps and tables make much of the textual information available at a glance: there is an excellent bibliography, some photographs of marginal usefulness and many charming drawings by John Busby. Overall, a valuable book of a remarkable survey. Other nations please copy.

In turn, Britain, overrun by mink and, to some opinions, by Canada geese, might do

John Busby

well to copy Western Australia in evolving a firm policy to control importation and translocation of potentially harmful wildlife. As a step in this direction, the States Agriculture Protection Board initiated a study which became, in the hands of John Long, the author of *Introduced Birds of the World* (David & Charles, £15), a comprehensive review of every known avian introduction worldwide. He found records involving 425 species and for each describes its natural and introduced range, the history and outcome of its introduction, and discusses its potential for damage. And damage there has been — extinctions of native species through competition and hybridization, substantial losses of agricultural and fruit crops and, possibly, the transfer of diseases and parasites. Western Australia is to be congratulated on the book: would our own agriculture departments were as wise. □

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The dissent of woman

Andrew Hill

On Becoming Human. By Nancy M. Tanner. Pp.373. ISBN hbk 0-521-23554-5; ISBN pbk 0-521-28028-1. (Cambridge University Press: 1981.) Hbk £20, $29.95; pbk £6.95, $10.95.

CHIMPANZEES are more like human beings than they appear. Linnaeus — the type specimen of taxonomists — originally classified the beasts in the human genus as *Homo troglodytes,* and scientists have continued to stress the similarities. *On Becoming Human* follows this tradition by proposing the chimpanzee as a model for the common ancestor of apes and man.

The book possesses a number of attractive features. Some of the latest evidence of affinity comes from work on proteins, and here we have a useful summary. There is also much on chimpanzee behaviour, emphasizing the

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role of females in their societies, and in evolution as well. Importance is attached to social factors in evolutionary change, to cooperation rather than competition, and to the probable significance of plants both as food and as material for tool making. This information, along with details of fossil finds, is put together to provide a story of human evolution that more or less fits with what is known. But after all, so little *is* known — although this has never been a drawback for theorists in evolutionary anthropology.

In addition, the book has unfortunate aspects. Palaeontological objections to assessments of phyletic divergence times based upon protein work are not thoroughly explored. The description of chimpanzee behaviour is not adequately set in the context of broader primate ethology, and is needlessly anecdotal rather than being presented in a rigorous scientific manner. Given the aim to show the role of female sociability as a force in human evolution, the chimpanzee female is not a particularly good analogue, since its social network is more restricted than those of many other higher primates. Further, there are things to object to in the palaeoanthropological sections as well, such as an idiosyncratic view of stratigraphy.

Perhaps worst, the text itself has been seriously affected by the contemporary problem of inflation. The material could have been better organized, and the style is very repetitious (with frequent parenthetical interruptions) and often says the same thing twice (or more). The maps are so reduced that it is impossible to see whether they would convey information even if larger, and the illustrations are inappropriately cute and sketchy for work of whatever sort this is — indeed it is difficult to guess for whom the book is intended.

Models have a limited value; plausibility is about the only thing they satisfactorily demonstrate. They raise questions and illustrate possibilities, for it is likely that transitional hominids were at least as complex as chimpanzees. But they can produce a confining and chauvinistic outlook. Tanner appears to reject anything that smacks of reductionism, but surely more reliance can be placed upon conclusions derived from a larger taxonomic base, from a method that aims to detect general principles. What are the consistent social correlates of a long life, for example, or a long period of infant dependence, in a wider range of mammals?

Anthropological beliefs are the product of their time, and while attempting to expose this and the role of nineteenth-century British male prejudice, I feel that Tanner falls into much the same trap by infusing her treatment with the equally parochial attitudes of some contemporary American women. □

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Animal behaviour from A to W

Thomas E. McGill

The Oxford Companion to Animal Behaviour. Edited by David McFarland. Pp.657. ISBN 0-19-866120-7. (Oxford University Press: 1981.) £17.50. To be published in the US early next year.

THE 1973 Nobel Prize for Physiology and Medicine was awarded to the animal behaviourists Karl von Frisch, Konrad Lorenz and Nickolaas Tinbergen. That event was, of course, the major landmark in public recognition of the discipline. But it is still gratifying that Oxford University Press has selected animal behaviour as the subject of the first of their scientific "Companion" series.

The book "has been designed as a non-specialist introduction to the study of animal behaviour". It contains an alphabetical series of 227 articles by McFarland and 71 other contributors. The essays, from Abnormal Behaviour to Wildlife Management (no Zoos?), range in length from a few lines — on, for example, immobility and gregariousness — to 18 pages on the history of animal behaviour. Many of the articles include excellent illustrations. The volume ends with a bibliography of 146 books and with indices of both the common English names and the scientific names of animals. Each index indicates the articles where a particular species is mentioned.

Six articles are devoted to people — the three Nobel laureates plus Charles Darwin, Ivan Pavlov and B. F. Skinner. The essays contain a brief biography and a discussion of the contributions of these luminaries.

While the book is designed for the layman, many will find it useful in teaching. For example, a two-paragraph article on vacuum activities provides some interesting examples and discusses how these behaviours might occur through stimulus generalization. A cross-reference leads to an article on generalization, containing other cross-references, and also a reference to the bibliography.

Aside from the inevitable unevenness of an edited work, the book has a major deficiency. It contains no listing of the titles of the articles, neither in a table of contents nor in an index. Thus one is left to guess the title of a particular subject. For example, while a scientific society and an international journal are called "Behaviour Genetics", there is no listing under that topic. Instead, the material is found under Genetics of Behaviour. There are no articles entitled Biological Clocks, Biorhythms or Circadian Rhythms; the relevant material is discussed in the contributions on clocks and rhythms. There is no entry for sociobiology (surely this topic deserves at least a cross-reference).

Guessing and page-turning proved mildly annoying, so I made my own list of the articles. I would advise others to do the same since this list proved a great aid in finding material and in browsing amongst unfamiliar topics. Certainly, there is treasure in this valuable book, but much of it is buried. □

Thomas E. McGill is Chairman and Hales Professor of Psychology at Williams College, Williamstown, Massachusetts. He is editor of Readings in Animal Behavior, *now in its third edition.*

The IQ agony aunt

Steve Blinkhorn

Straight Talk About Mental Tests. By Arthur R. Jensen. Pp.269. ISBN US 0-02-916-440-0; ISBN UK 0-416-32300-6. (Free Press, New York/Methuen, London: 1981.) $12.95, £8.95.

WITH a publisher's blurb that would do credit to a headache remedy advertisement, and a shoulders-squared, plain brown wrapper of a title, Jensen's latest offering is a curious mixture. Part textbook, part highbrow agony column, it proclaims itself to be a plain man's guide and largely eschews mathematical formulae and reference to source material. But despite the obvious good intentions the book turns out to be no more than a competent rehash, lacking the brash vigour of Eysenck's brand of popular psychology and failing to compensate with novelty of argument or perspective.

What, after all, is there new to say about mental tests? Not enough, apparently, to merit a whole book, since the predominant theme of this one is the primacy and genetic determination of general intelligence, rather than the mental tests of the title. This is not to say that there is any evidence of lack of competence. As in *Bias in Mental Testing* (Free Press/Methuen, 1980), clearly the elder brother of this volume, Jensen shows considerable mastery of the issues. And when it comes to the evaluation of the role of IQ measurement for practical purposes, there is no evidence of support for the kind of gee-whizzery and technical witch-doctory that mental tests can attract. It needs to be said by such as Jensen that tests are overused and often misused, but not thereby rendered useless, and he makes the point clearly and sensibly.

It is all the more difficult, then, to understand why he finds it necessary to pick continually at the scab of black–white IQ differences, why two of the six titles in the select bibliography concern race, indeed why he could not confine himself to the matter of mental tests, the jargon and technology of which are as often misunder-

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stood amongst psychologists as in the world at large.

In the final chapter, switching from solid, textbook prose to a question-and-answer format suggesting the world's most degree-laden agony aunt, Jensen treats the reader to a glimpse into his postbag.

- "My father is a world-recognized genius. Yet I am sure he would flunk any IQ test. What do you think of that?"
- "What can I do for my child to raise his IQ?"
- "Is our national IQ declining?"

No doubt these are genuine extracts from letters the originals of which are available for inspection at the author's premises. The format of this chapter certainly conveys the impression of an attempt to provide a plain man's guide, but at the same time shows up the failure of the enterprise as a whole. For what Jensen has done is not to strip off inessential technicalities to reach a wider audience, but to present himself as the man in the white coat and invite confidence in his knowledge and expertise. He lacks neither, but the suspicion always lurks that he is too much of an interested party to be wholly dispassionate. □

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The naturalist's art

T.R. Halliday

ART and natural history have always been closely associated. But, over the past hundred years, illustrations have fulfilled a variety of functions in the natural history literature; the purpose of some is simply to aid identification, of others to be purely decorative and of yet more to encapsulate some aspect of the natural way of life of the creature portrayed. A number of recently published books not only enable us to trace the development of art in natural history from the nineteenth century to the present day, but to see how different artists have been concerned with one of these aims more than another.

A new, selected reprint of the Reverend F. O. Morris's *British Birds,* first published in parts between 1850 and 1857, serves to show how far natural history illustrations have progressed in the past 130 years. This reprint (Webb & Bower/Holt, Rinehart & Winston; £20, $40) has a brief introduction by Tony Soper, describing the aims of Morris's work and its impact on the public of his time. The coloured engravings by A.F. Lydon often bear only superficial resemblance to their subjects, the poses being wooden and unnatural; they can have been of little value as guides to the identification of living birds in the field. The worth of a reprint such as this is entirely historical. The book is of no use as a guide for a modern bird watcher but does, through its text, give an insight into the understanding that early ornithologists had of birds.

An artist generally recognized as one of the early masters of bird painting was John Gould, who died in 1873. A beautifully produced volume, *John Gould's Birds* (A&W Publishers, New York; $39.95 until December 1981, $50 thereafter), reproduces a rich selection of his finer work. Maureen Lambourne's introduction provides a biographical sketch of this productive and much-travelled artist. Gould's paintings are essentially decorative and are mostly in the form of vignettes, with the subject posed against foliage or a hint of landscape. Some of his work was remarkably ambitious, such as his painting of a barn swallow feeding her young in flight. His cock pheasant, far from being shown in its customary splendour, is portrayed lying dead in a snare. Gould's scrupulous attention to detail shows, not only in his birds, but also in the plants with which many are framed. Though his pictures are often stilted and contrived by today's standards, Gould's skill and accurate observations make this book a pleasure to look through.

The latest tribute to Archibald Thorburn, who died in 1885 (*Thorburn's Landscape* by John Southern, published by Elm Tree Books at £12.50), reproduces many of his larger paintings, in which as much attention is devoted to the landscape as to the birds. Most of the paintings are of game birds and are set in moorland, mountains and forest. Thorburn's skilful treatment of light, dark and colour beautifully evokes the chill of autumn or the bleakness of a snow-covered mountain side. Like Gould, Thorburn sometimes attempted a painting more ambitious than a posed group of birds, such as a peregrine killing a mallard in flight or a group of ptarmigan cowering as an unseen golden eagle casts its shadow over them. His rich use of colour and intimate knowledge of the habits of birds make this a fine book.

Following his death in 1979, a number of books have been published that display the work of Charles Tunnicliffe. Robert Gillmor edits and introduces the latest of them, *Sketches of Bird Life* (Gollancz; £10.95). Most of the book is devoted to Tunnicliffe's field sketches that provided the raw material for his major paintings. An interesting feature of this book is the way that it shows how the artist developed his work from field sketch to finished painting; more examples of this would have been welcome. The sketches, mostly in pencil or watercolour, reveal that Tunnicliffe was not only a supreme artist, but also a fine ethologist. Behavioural interactions between birds are beautifully recorded, both visually and in the annotations that appear on many of the sketches. Tunnicliffe's extreme devotion to his art was remarkable, as shown by his meticulous paintings of dead birds which, together with his field sketches, he used in the creation of his finished paintings.

The work of a new, living artist is presented in *Keith Brockie's Wildlife Sketchbook* (Dent/Macmillan, New York; £9.50, $19.95). Working in Scotland, Brockie has carried on the Tunnicliffe tradition, never missing an opportunity to record a scene, a flower, a piece of driftwood or a dead bird, as well as patiently sketching living animals from a hide. Working mostly in pencil, crayon and watercolour, Brockie has the gift of being able to catch the essence of an animal, whether it is a bird, a fish or a rabbit. All that this attractive book lacks are some examples of how the artist might use his sketches to produce more formal paintings.

In sharp contrast, the contemporary American artist Robert Bateman specializes in huge paintings, beautifully

Three redstarts (*Phoenicurus phoenicurus phoenicurus*) painted by John Gould.

reproduced in *The Art of Robert Bateman* with text by Ramsay Derry (Viking/Allen Lane; $40, £20). Bateman's breathtaking pictures are not pretty portraits of animals. His aim is clearly to emphasize the close relationship between an animal and its environment and, in many of his paintings, the animals seem to take second place to the landscape. There is much variety in the scope of his view, from panoramic vistas of Arctic tundra or the East African plains to restricted glimpses up into a tree or through the window of a derelict house. Bateman is an artist of many skills. Not only are his birds and mammals painted with exquisite accuracy of detail and posture, but his use of light and colour perfectly captures the icy cold of the tundra and the heat of the Serengeti plain. These are remarkable paintings that repay long and careful study, and this book stimulates a keen desire to see the original works. □

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